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THE UNIVERSITY OF ALBERTA

PROPRIOCEPTIVE INPUT TO THE LOCUST FLIGHT OSCILLATOR

by

© DAVID N. REYE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

EDMONTON, ALBERTA

SPRING 1986

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ABSTRACT

The influences of electrical stimulation of the wing stretch receptor (SR) afferents on the flight rhythm and on the activity of single identified thoracic interneurons were examined in semi-deafferented and deafferented preparations of *Locusta migratoria*.

Triggering bilateral forewing SR stimulation from the motor output (depressor) caused a rapid increase in the flight frequency and a marked prolongation of the flight sequence duration in semi-deafferented preparations. These effects were critically dependent on the timing of the stimulation in the flight cycle. Stimulation had to occur close to the depressor phase of the flight cycle. Stimulation presented midway between depressor bursts had no obvious effects on either the frequency or duration of flight sequences. Single SR stimulus trains presented at different times in the flight cycle reset the flight rhythm when presented close to the time of the depressor spike. Repetitive presentation of SR stimulus trains could entrain the rhythm. During entrainment, the onset of each stimulus train was close to the onset of depressor activity. These results showed that the SRs have strong phase-dependent influences on the flight rhythm generator. They disagree with previous studies that the SRs had only a slow tonic influence. Stimulation of segmental pairs of SRs could entrain the flight rhythm in deafferented preparations. In deafferented preparations, the range of frequencies over which entrainment could be obtained was reduced compared to that seen in semi-deafferented animals. The maximum frequency reached with triggered stimulation was less than that seen in semi-deafferented preparations. Stimulation of an ipsilateral pair of SRs could increase the frequency when stimulated close to the depressor phase. However, this pair of SRs could usually not entrain the deafferented flight rhythm.

Intracellular neuropil recordings were made from identified thoracic interneurons and motoneurons to examine their response to SR input. Constant, short-latency excitatory postsynaptic potentials (EPSPs) occurred in depressor motoneurons following each SR afferent spike. In contrast to earlier findings, the SRs did not evoke hyperpolarizing potentials in elevator motoneurons. SR stimulation during deafferented flight activity evoked long latency

influences on motoneurons. There was a reduced repolarization of depressors and a reduction of the depolarization of the following elevator burst so that fewer elevator spikes occurred in each cycle.

Twenty-two interneurons were identified in which short-latency EPSPs followed each SR afferent spike. Where tested, all interneurons received convergent input from more than one SR. The majority of connections were to depressor interneurons but also elevator interneurons, a non-rhythmic interneuron and a tonically active neuron received EPSPs. One interneuron was found which received a longer latency IPSP from all four SRs. SR stimulation during flight caused major alterations in the activity of some of these interneurons, including increased burst frequency, prolonged duration of bursts, abolition of activity and alteration in the phase of activity.

The SRs evoked EPSPs in interneurons which fired tonically during flight. The properties of these neurons indicated they were involved in the initiation and maintenance of the flight rhythm. The response of these neurons to SR stimulation during flight was examined to investigate their role in the SRs' effects of increasing the flight frequency and prolonging the duration of flight sequences.

The major conclusions from this work were that (i) the wing SRs are important elements in the flight rhythm generator, influencing the frequency and duration of flight sequences; (ii) the activity of rhythmically active neurons in a deafferented rhythm can be markedly altered in the presence of proprioceptive feedback and (iii) there is a separate neuronal system providing an unpatterned drive to the locust flight rhythm generator.

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I. INTRODUCTION

A. ORGANIZATION OF RHYTHMIC MOTOR SYSTEMS.

A major goal in neurobiology is to understand how, through neuronal interactions, organisms produce successful and adaptive movements. To this end, rhythmic behaviors have received a great deal of attention. Many fundamental movements are of this type - locomotory, respiratory and feeding movements being the most common. These behaviors have the experimental advantage that the same (or similar) motor pattern is repeated over and over again and can often be produced in highly dissected preparations.

A finding of importance for our understanding of the neural organization subserving rhythmic movements is central rhythm generation. Early ideas suggested that rhythmic movements arose from sequential activation of sensory reflexes. That is, each phase of a movement excited peripheral sensory receptors which then activated the following phase of the movement and so on. This hypothesis predicted that movements could not be generated without sensory feedback from the moving appendages. There are now many cases where this hypothesis has been tested by depriving animals of phasic sensory feedback by deafferentation and/or deefferentation. It has been found that such deafferented preparations can still produce a rhythmic motor output. Experiments of this type have been performed in a large number of invertebrate and vertebrate species and central rhythm generation appears to be a common feature of most, if not all, rhythmic behaviors so far examined (Delcomyn 1980). This phenomenon demonstrates that within the central nervous system there are neural circuits, which, when activated can produce an organized rhythmic output pattern.

The most common term used to describe these circuits is "central pattern generator" (CPG). However, the precise neural elements encompassed by this term have not been defined consistently. The most common usage is to define the elements of the CPG as those neurons which, by virtue of intrinsic properties and interconnections are responsible for rhythm generation itself (Delcomyn 1980, Kristan 1980). It appears to be common (though not always

true) that rhythm generation is produced by specific interneurons and that motoneurons and other interneurons are simply driven by this network (Kristan 1980, Selverston 1980, Selverston and Moulins 1985). However, the actual pattern of motor output (as seen in nerve root or EMG recordings) is a product not only of the neurons generating the rhythm but also of non-rhythm generating neurons. Thus, Grillner and Wallén (1985) include in the CPG all the neurons responsible for the output pattern but exclude neurons with an initiating function or with a purely output function (e.g. motoneurons). Pinsker and Ayers (1983),however, refer to the CPG as all neurons responsible for the output pattern including initiating neurons and motoneurons. In my view, it is useful to distinguish between rhythm generating and non-rhythm generating neurons. Therefore, I will call the system of rhythm generating neurons the central rhythm generator or central oscillator. The term, 'central pattern generator', will encompass all the rhythmically active neurons responsible for the deafferented output pattern. Included in this latter category are the motoneurons. The term does not include neurons which have a purely initiating function.

Many rhythmic behaviors are episodic in that they are expressed only at particular times. Thus mechanisms must exist by which the behavior is initiated, maintained and turned off. It has been found that certain individual neurons or groups of neurons can initiate rhythmic activity when stimulated. These neurons are commonly termed 'command' neurons (Wiersma and Ikeda 1964, Kupfermann and Weiss 1978). Importantly, it has been found that this stimulation does not need to contain timing cues to elicit a patterned motor output. For example, unpatterned stimulation has been found to elicit swimmeret beating in crustaceans (Wiersma and Ikeda 1964, Davis and Kennedy 1972) and singing in crickets (Bentley 1977), walking in deafferented cats (Grillner and Zangger 1974) and swimming in fish (Kashin et al 1974) and turtles (Lennard and Stein 1977). This has suggested that in conjunction with the CPG there is a separate neuronal system which provides a tonic drive to the CPG. However, while patterned stimulation may not be necessary to elicit rhythmic activity, it is not clear that the neurons able to initiate rhythm generation do not also provide phasic information. In the

Pleurobranchaea feeding system, neurons which can initiate the feeding rhythm are also rhythmically active and can reset the rhythm (Gillette et al 1978, Davis and Kovac 1981). These workers have suggested that there may not be separate neuronal systems for rhythm initiation and pattern generation (Davis and Kovac 1981). Neurons which are rhythmically active and can also initiate rhythms have been described for swimming in *Tritonia* (Getting 1977, Getting and Dekin 1985), feeding in *Lymnaea* (Rose and Benjamin 1981) and swimming in the leech (Weeks 1982). Weeks and Kristan (1978) have also described swim-initiating neurons in the leech whose properties suggest that they may provide a more-or-less tonic drive to the swim oscillator. These neurons (the 204 neurons) are active only during a swim, they initiate swimming when depolarized and are capable of slowing, or stopping swimming when hyperpolarized. While they do show a weak modulation in phase with the swim rhythm, it does not appear that this provides any significant timing information to the swim oscillator (Weeks 1981). To date, these data on the 204 neurons in the leech swimming system provide the best example of separate systems for the initiation and patterning of a rhythmic activity.

Although rhythmic motor patterns can be generated in the absence of sensory input, it is clear that sensory feedback is important in regulating movements. Virtually all motor systems possess proprioceptors which are strongly excited by the animal's own movements (Sherrington 1906). The role of proprioceptive feedback in the generation of rhythmic motor patterns has been studied with a variety of experimental techniques. One can compare the output of the deafferented nervous system with that seen in the intact behavior. An ongoing movement may be perturbed or a movement may be experimentally imposed on the animal (e.g. with a treadmill). The normal firing of a proprioceptor can be altered by lesioning or by mechanical or electrical stimulation. In some behaviors, deafferentation leads to little or no change in the motor output, such as swimming in the *Xenopus* embryo (Kahn and Roberts 1982) and the turtle scratch reflex (Robertson et al 1985). Grillner and Wallén (1985) have also emphasized the similarities between the deafferented and intact motor output pattern in fish swimming and cat walking. The hindlimb spinal segments of a walking cat still produce a complex pattern of

activity similar to the pattern in intact animals (Grillner and Zangger 1979). In the lamprey and dogfish, the patterns of swimming activity before and after deafferentation appear very similar, although in the lamprey there may be some reduction in mean frequency after deafferentation (Wallén and Williams 1984). While the CPGs for walking in the cat and swimming in the lamprey and dogfish do produce a similar motor output in the absence of sensory information, very strong peripheral influences have also been demonstrated. For example, in the cat, hip proprioceptors play a prominent role in phase-switching between extension and flexion (Andersson et al 1981) and also reinforce the muscle activity during the movement (Andersson and Grillner 1981). The onset of the swing phase is also inhibited if the hindlimb extensor muscles are not unloaded (Pearson and Duysens 1976). Stretch receptors in the dogfish and lamprey also act to limit the amplitude of movements and to reinforce the m[illegible] activity underlying the movement (Grillner and Wallén 1977, 1982, Grillner et al 1981). In other vertebrates, more obvious changes in motor output have been reported after deafferentation. In anuran larvae, there is a loss of rostro-caudal phase lag after removal of sensory input (Stehouwer and Farel 1980) and in newt walking a large increase in cycle period can be seen (Székély et al 1969). In the cat scratch reflex, deafferentation causes an increased amplitude of movement and an inability of the animal to place its limb in the correct position (Deliagina et al 1975).

Deafferentation can also lead to dramatic changes in motor output in some invertebrates. In some cases, the deafferented motor pattern could be related to any of several different intact behaviors (e.g. rocking, searching, grooming in the stick insect, Bässler 1983; rejection, regurgitation and swallowing in *Pleurobranchaea*, McClellan 1982). Increases in cycle period following deafferentation have been reported (e.g. locust flight, Wilson 1961; insect ventilation, Lewis et al 1983; crayfish walking, Clarac and Sillar 1985). In some crustaceans, contact of the legs with the ground is necessary to obtain normal intersegmental co-ordination (Pasztor and Clarac 1983, Clarac and Sillar 1985). Changes in interlimb co-ordination are also seen in insect walking following amputation of two or more limbs (Wendler 1966, Delcomyn

1971).

A major question for rhythmic motor behaviors is how the central oscillator and sensory input are integrated together to produce the motor output seen in intact animals. A first approach to this problem is to find those sites within the nervous system where proprioceptors act. It is possible to distinguish between two sites of action by analyzing how proprioceptive stimulation modifies the motor output. If proprioceptive stimulation affects the amplitude or phasing of a motoneuron burst without influencing the timing of the rhythm generator then it may be inferred that the proprioceptor is acting on motoneurons or non-rhythm generator interneurons. If the cycle period of the rhythm is altered without a compensatory change in the period of subsequent cycles, then the rhythm generator itself must have been influenced (Perkel et al 1964, Aschoff 1965, Pinsker 1977a). Alternately, a series of sensory stimuli can be given at a rate different from the intrinsic rhythm. If the rhythm adopts the frequency of the sensory stimulus with a preferred temporal relationship between the stimulus and the rhythm, then the rhythm is said to be entrained by the stimulus and a direct action on the central rhythm generator may be inferred (von Holst 1939, Perkel et al 1964, Enright 1965). Entrainment not only implies resetting of the rhythm generator but also that the magnitude of the resetting should be dependent on the time in the cycle at which the stimulus is given (Perkel et al 1964, Enright 1965, Pinsker 1977b). By such techniques phase-dependent influences of proprioceptors on rhythm generators have been demonstrated in a number of systems. These include ventilation in mammals and insects (Wyman 1977, Farley and Case 1968), swimming in the lamprey and dogfish (Grillner et al 1981, Grillner and Wallén 1982), walking in the cat (Andersson and Grillner 1981, 1983), insect flight (Wendler 1974, Möhl and Nachtigall 1978) and lobster feeding (Nagy and Moulins 1981).

To date, most studies of the cellular basis of rhythmic behaviors have concentrated on central neurons, particularly with regards to the mechanisms of rhythm generation (Kristan 1980, Selverston and Moulins 1985). Much less is known of the cellular mechanisms by which proprioceptors influence a central rhythm generator. The most detailed knowledge at the

cellular level has come from work on mammalian respiration (Cohen 1979). Unfortunately, the mechanisms of rhythm generation are still unknown for this system, although influences of pulmonary stretch receptors on postulated inspiratory off-switch neurons have been found (Cohen 1979, Cohen and Feldman 1984).

In insects, the importance of sensory feedback has been established for several different behaviors (flight; Wendler 1983, Heide 1978; stridulation, Elliott and Koch 1983; walking, Wendler 1966, Bässler 1983, Delcomyn 1985). Proprioceptive connections to motoneurons have been described for each of these behaviors (Burrows 1975, Elliott 1983, Pearson et al 1976). Connections from leg receptors to interneurons in locusts have been described in detail by Siegler and Burrows (1983, Burrows 1985). However, these studies used quiescent preparations so that the influence of a central rhythm generator on these neurons is unknown. The most progress in understanding the cellular basis of an insect CPG has come from work on locust flight (Robertson and Pearson 1982,1983,1985). I have used this behavior to examine the influence of proprioceptors on a central pattern generator at the cellular level. The particular proprioceptors used were the wing stretch receptors. The physiology and anatomy of the receptor afferents is such that a reasonable facsimile of their intact discharge can be selectively elicited by electrical stimulation. This enabled the comparison of the activity of single identified interneurons driven solely by the central oscillator to that seen in the presence of proprioceptive input. The following section describes the locust flight behavior and considers previous work in relation to the problem of central-peripheral interaction in this animal.

B. LOCUST FLIGHT

Wing Movements

The locust has two pairs of wings, an anterior pair (the forewings) located on the dorsolateral mesothorax and a posterior pair (the hindwings) located on the metathorax. In free-flying locusts the wingbeat frequency is over 22 cycles per second (Baker et al 1981).

Tethered locusts fly at a slightly lower frequency, usually in the range of 17 - 20 cycles per second (Weis-Fogh 1956a, Baker et al 1981). The wingbeat frequency is the major factor governing flight speed (Gewecke 1975, Baker et al 1981). During the upstroke the wings move upward and backward relative to the body and during the downstroke the wings are brought downward and forward (Jensen 1956). There is a phase lag between the two segments, such that the hindwings lead the forewings. The absolute amount depends on the phase of the wingstroke under consideration. On average they lead by approximately 5 msec (ca.9% of cycle time) (Weis-Fogh 1956a). In order to progress through the air at a constant velocity, the locust has to maintain a lift equal to the gravitational pull and also to produce a thrust equal to the aerodynamic drag. Most of the power of flight is involved in generating lift (Jensen 1956). Most of the lift (ca.80%) is generated during the downstroke, particularly during the bottom half (Jensen 1956). The hindwings, because of their bigger surface area produce approximately 70% of the lift generated during a wingstroke and approximately 75% of the thrust (Jensen 1956). The hindwings describe a quite constant path and move synchronously while the forewings show more variability both in their path and in synchrony between the two sides (Weis-Fogh 1956a, Baker and Cooter 1979). In addition to the [illegible]up and down movements, the angle of attack of the wing changes during the stroke, most notably there is a distinct pronation of the wing at the top of the upstroke and during the downstroke (Jensen 1956).

The most important mechanism by which the locust maintains flight stability and initiates steering manoeuvres appears to be via control of the angle of attack of the wings, particularly of the forewings (Weis-Fogh 1956a, Wilson and Weis-Fogh 1962, Gettrup and Wilson 1964, Dugard 1967, Baker 1979a). For example, to steer to one side, the locust increases the forewing pronation on the side of the turn which reduces the angle of attack of that wing. This reduces the lift and thrust on that side so that there will be both a roll and yaw movement to the side of the pronated wing (Dugard 1967, Baker 1979a).

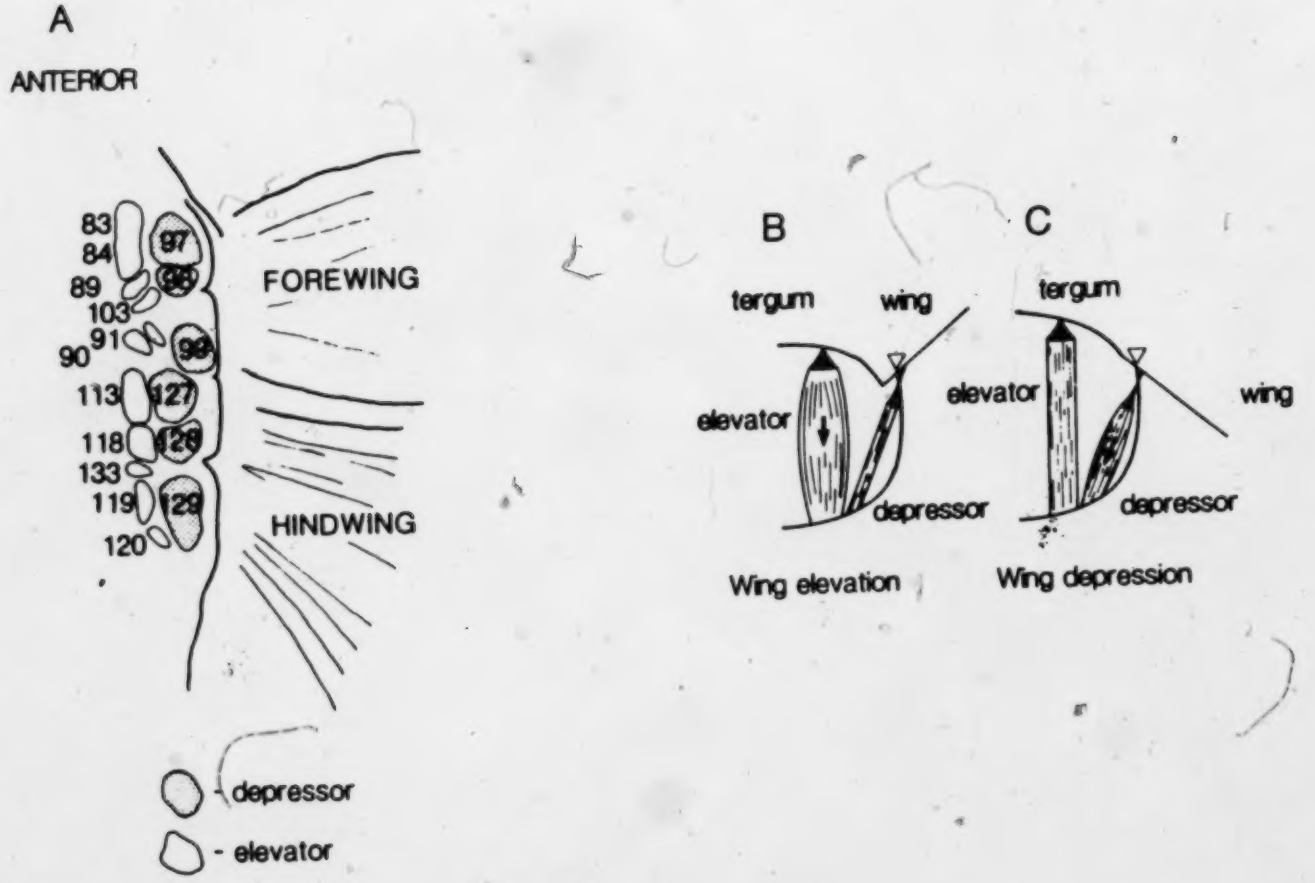
Muscular Control

The muscles controlling the wing movements can be divided into three groups : elevators, depressors and the accessory muscles. The depressors can be further subdivided into the direct depressor muscles (first basalar, second basalar and subalar) and the indirect depressors (the dorsal longitudinals). Each set of muscles is repeated in both mesothoracic and metathoracic segments. The arrangement of the elevator and direct depressor muscles for one side of the animal is shown in Figure 1. Each muscle is aligned dorsoventrally. Both elevator and depressor muscles form a rostrocaudal row, the elevators located medially to the depressors. The elevators insert dorsally on the tergum and raise the wing indirectly by compressing the thorax in the dorsoventral direction (Fig. 1b) Thus the elevators do not directly control the wing profile during the upstroke, this being determined by aerodynamic and elastic forces in conjunction with activity in the accessory muscles (Pfau and Nachtigall 1981). On the other hand, the direct depressor muscles insert onto the sclerites of the wing hinge and, by changes in their relative timing (in conjunction with the accessory muscles) can affect the wing twisting during the downstroke (and hence the lift generated) (Zarnack and Möhl 1977, Baker 1979b). In addition to the direct depressors, there are the rostrocaudally aligned dorsal longitudinal muscles which depress the wing indirectly by compressing the thorax in the rostrocaudal direction, thereby raising the tergum.

The third group, the accessory muscles have only recently received attention following the functional anatomical investigation of Pfau (1978a). These are small muscles originating high on the pleuron which insert onto the sclerites of the wing hinge. It had previously been thought that wing twisting was controlled by an antagonistic action between the basalars and subalar muscles, with the basalars acting as the pronators and the subalars as supinators (Wilson and Weis-Fogh 1962). More recent work (Pfau 1978a, Pfau and Nachtigall 1981, Heukamp 1984) now suggests that wing twisting is regulated by the amount of tension in the pleuroalar muscles as well as by changes in the relative timing of the basalar and subalar muscles.

Figure 1. The muscles responsible for rhythmic wing movements. A: Longitudinal section through the thorax, as would be seen from the ventral side. Elevator muscles (open) and the direct depressors (stippled) form a rostrocaudal row. with the depressors located laterally to the elevator muscles (adapted from Möhl 1985a). B and C: Schematic illustration of the principles by which the flight muscles move the wings in orthopterans. The elevators insert on the tergum and raise the wing indirectly by compressing the thorax dorsoventrally (B). The direct depressors insert on the sclerites of the wing laterally to the pivot point (triangle). Wing depression is a result of a direct action of the depressors which pull the wing downward. This is further aided by elastic recoil of the thorax upon relaxation of the elevator muscles which raises the tergum (C). The indirect depressors (the dorsal longitudinal muscles) add power to the downstroke by raising the tergum indirectly by compression in rostro-caudal direction (adapted from Chapman 1971). The numbering of the muscles is that of Snodgrass (1929). The corresponding names of the muscles are given below.

	MESO.	META.	
ELEVATORS:	83	113	First tergosternal
	84		Second tergosternal
	89	118	Anterior tergocoxal
	90	119	First posterior tergocoxal
	91	120	Second posterior tergocoxal
	103	133	Tergotrochanteral
DEPRESSORS:	81	112	Dorsal longitudinal
	97	127	First basalar
	98	127	Second basalar
	99	129	Subalar


A
ANTERIOR
83
84
89
103
91
90
113
118
133
119
120
97
95
99
127
126
129
FOREWING
HINDWING
depressor
elevator
B
tergum
wing
elevator
depressor
Wing elevation
C
tergum
elevator
wing
depressor
Wing depression

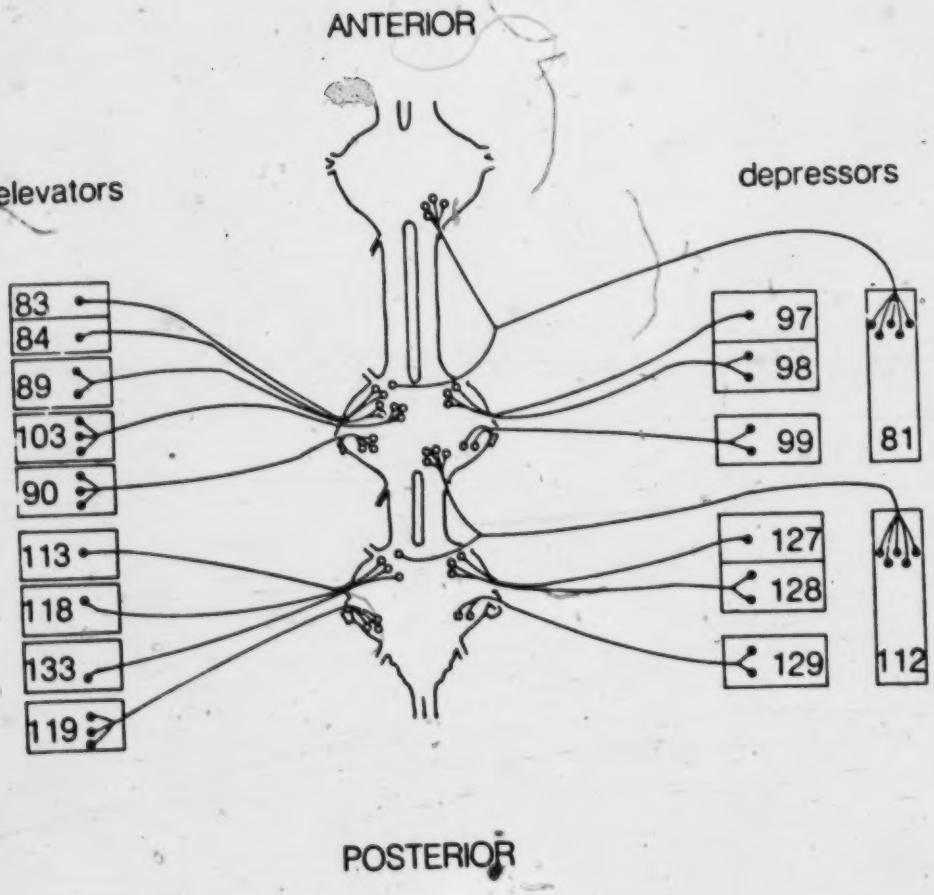
The motoneurons innervating the elevator and direct depressor muscles are located in the ipsilateral hemiganglion of the same segment as the muscle (Bentley 1970, Burrows 1973, Tyrer and Altman 1974). That is, motoneurons controlling the mesothoracic flight muscles are located in the ipsilateral mesothoracic hemiganglion and those of the metathoracic flight muscles are located in the ipsilateral metathoracic hemiganglion. The location of these motoneurons is illustrated in Figure 2. The indirect depressors, the dorsal longitudinals, differ in several respects. Each muscle is innervated by five motoneurons (Neville 1963). One motoneuron lies in the same segment as the muscle but with a contralateral cell body while the other four motoneurons are located in the next anterior ganglion to the segment of innervation (i.e. the prothoracic ganglion for the mesothoracic dorsal longitudinals, the mesothoracic ganglion for the metathoracic dorsal longitudinals).

Nervous Control

Initiation and Maintenance

On the locust's head are several fields of wind-sensitive hairs which are responsive to wind moving in a particular direction (Weis-Fogh 1949, Guthrie 1964, Camhi 1969a, Sviderskii 1967). The sensory cell innervating each wind hair discharges with a slowly adapting spike train (Sviderskii 1967, Camhi 1969a). Most wind hair primary afferents descend only as far as the suboesophageal ganglion (Camhi 1969b) but a small number project to the prothoracic and mesothoracic ganglia (Tyrer et al 1979). The wind hair afferents excite descending wind interneurons which project to the thoracic ganglia (Camhi 1969b). Several of these wind interneurons have been identified (Bacon and Möhl 1979, Simmons 1980, Reichert et al 1985) and these have been found to also receive input from other sense organs such as the compound eyes, the antennae and ocelli. Information from the wind hairs, ocelli, compound eyes and antennae are used by the animal to detect changes in pitch, roll and yaw and are important in evoking steering reactions and maintaining stability (Gettrup and Wilson 1964, Goodman 1965, Taylor 1981, Cooter 1979,

Figure 2. Location of motoneurons innervating flight muscles in the locust. The innervation of elevator muscles is indicated to the left, that of the depressor muscles to the right. In general, muscles are innervated by neurons ipsilateral to and in the same segment as the muscle. The motoneuron axons exit the ganglia via nerves 3 and 4. The exception are the motoneurons to the dorsal longitudinal muscles (m81). The dorsal longitudinal motoneurons are divided in two groups and leave the CNS via nerves 6 and 1. Four of the five motoneurons are located in the next anterior ganglion to the innervated muscle. The fifth motoneuron has a contralateral cell body located in the same segment as the muscle. (After Tyrer and Altman 1974 and Burrows 1975).


ANTERIOR
elevators
depressors
83
84
89
103
90
113
118
133
119
97
98
99
81
127
128
129
112
POSTERIOR

Reichert et al 1985).

Sufficient conditions for the initiation and maintenance of flight in intact animals are a windstream to the head of the animal and loss of tarsal contact with the ground (Weis-Fogh 1949, 1956b). An important mechanism for maintaining flight, then is air movement over the locust's head as it flies. However, the head hairs are not necessary for flight activity as this can still be elicited by a windstream when the connectives from the brain are cut (Weis-Fogh 1956b). Sensory hairs on the thorax and wings may be responsible for maintained flight in these conditions (Weis-Fogh 1956b). Covering the head hairs with wax reduces the wingbeat frequency and the flight speed by approximately 25% (Gewecke 1975).

Flight can be initiated by wind over the head or a sudden loss of tarsal contact with a substrate (Weis-Fogh 1956b). In intact animals, flight is normally preceded by a jump which would elicit both these reflexes. In a set of behavioral experiments, Camhi (1969c) measured the time from a jump to wing opening. He found that the latency was too short for either wind on the head or loss of tarsal contact to be responsible and concluded that in intact animals, flight initiation involves central pathways linking the jump and flight systems.

Central Flight Oscillator

Wilson (1961), in a very influential paper, showed that a flight rhythm (consisting of alternating elevator and depressor bursts) was still expressed in the absence of any proprioceptive feedback from the wings. This central oscillator was located in the thoracic ganglia. The major difference that he observed between the deafferented and intact rhythms was that the frequency of the former was in the range of 6 - 12 Hz while in tethered flying animals the frequency is usually 17 - 20 Hz. Kutsch (1974) has further noted that lesioning the wing proprioceptors increases the cycle-to-cycle variability in period. The basic repeating unit in the central oscillator rhythm is firing of elevator motoneurons followed by discharge of the depressor motoneurons (Hedwig and Pearson

1984). Rhythmic depressor motoneuron depolarizations only occur following elevator motoneuron depolarizations. The duration of elevator activity increases with cycle period while the latency from the end of the elevator burst to the beginning of the depressor burst increases only slightly (Hedwig and Pearson 1984). Recenly, Robertson and Pearson (1982) developed a preparation for intracellular recording from flight interneurons during expression of the central flight rhythm. A large number of interneurons have been discovered and a neural circuit has been proposed to account for the generation of a sequence of depolarizations in elevator and depressor motoneurons (Robertson and Pearson 1985). An interesting result from this work is that movement of all four wings appears to be governed by one oscillator network rather than separate oscillators for each wing (Robertson and Pearson 1983).

Proprioceptors in Locust Flight

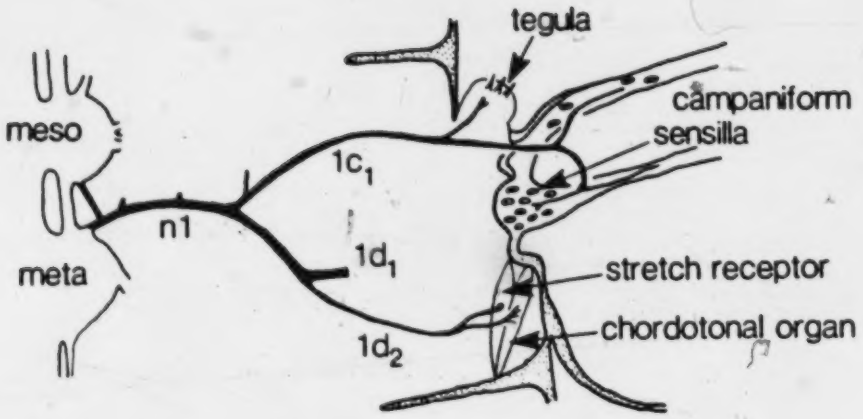
A lot of work on locust flight has concentrated on sensory influences involved in flight control. I consider here proprioceptive influences from the wing.

The known wing proprioceptors together with their sensory innervation are shown in Figure 3a. Only one hemisegment (hindwing) is shown but essentially this scheme is repeated on each side in both the mesothoracic and metathoracic segments. Four groups of proprioceptors are located on or close to the wing and can monitor various aspects of wing movement. Another proprioceptive group not shown in Figure 3 are the wind hairs on the head (see below). The proprioceptors of concern to this thesis are the wing stretch receptors (fig 3b). Firstly, I will describe other proprioceptors in the flight system, what is known about them and their proposed functions during flight.

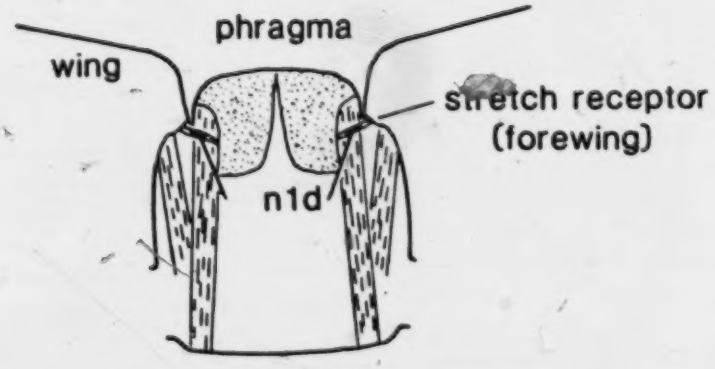
1. Head Hairs. A somewhat surprising group of phasic proprioceptors are the wind-sensitive hairs on the head. The movements of the wings, together with the nodding movements of the head during flight, generate wind currents around the head's hair fields which are modulated with the flight frequency (Bacon and Möhl 1979, 1983, Horsmann et al 1983). Mimicking these air currents by using a sinusoidally modulated wind-stream can

Figure 3. Sensory innervation of the locust wing and location of the forewing SRs. A: Schematic illustration of the innervation of the proprioceptors for one hindwing. This basic plan is the same for the forewings. All proprioceptive afferents from the wing enters the central nervous system via nerve 1 (meta.n1 for the hindwing, meso.n1 for the forewing). Four groups of proprioceptors are associated with each wing: the tegula and campaniform sensilla whose axons travel in n1c and the stretch receptor and chordotonal organ which travel in n1d. (adapted from Kien and Altman 1979). B: Location of the forewing stretch receptors. Cross-section through the mesothoracic coxae, viewed from the caudal end, showing the suspension of the SRs between the mesothoracic phragma and the wing base. (from Wendler 1983).

A


tegula
campaniform
sensilla
meso
1c1
n1
1d1
meta
stretch receptor
chordotonal organ
1d2

B


phragma
wing
stretch receptor
(forewing)
n1d

entrain the flight rhythm (Horsmann et al 1983). One of the descending wind interneurons modulated by these air currents is the tritocerebral giant (TCG, Bacon and Möhl 1979, 1983). Electrical stimulation of a TCG during flight can evoke a small reset of the flight rhythm (Bacon and Möhl 1983).

2. Tegula. The tegula is a small knob-like structure located at the anterior edge of the wing base (Kien and Altman 1979, Kutsch et al 1980). It is comprised of two sensory structures, an inner chordotonal organ with an outer covering of mechanoreceptive hairs (Kutsch et al 1980). Its structure and location suggested it may be excited toward the bottom of the wing downstroke when it would be contacted by the anterior membranous cuticle of the wing (Wilson and Gettrup 1963, Kutsch et al 1980). Recent recordings from the tegula nerve in intact tethered flying locusts (Neumann 1985) show a burst of activity occurring just before the firing of the elevator muscles which supports this interpretation of the phase of tegula activity in the wingbeat cycle.

The tegula sends about 80 axons to the central nervous system (Altman et al 1978, Kutsch et al 1980) which range in diameter from one to twelve micrometres. The hindwing tegula afferents project only to the metathoracic ganglion whereas the forewing tegula afferents enter the mesothoracic ganglion and also travel within the CNS to the metathoracic ganglion (Tyrer and Altman 1974). Kien and Altman (1979) stimulated the hindwing tegula in quiescent preparations of *Schistocerca gregaria*. Larger units from the tegula elicited EPSPs in a depressor motoneuron and an IPSP in a wing elevator motoneuron. The smaller fibres produced the opposite effects, namely inhibition of depressors and excitation of elevators. These results suggest two different influences from the tegula on the flight system. This may correspond to the two sensory structures contained in the tegula (Kutsch et al 1980). Neumann (1985) has stimulated the forewing tegula nerve in tethered flying *Locusta*. He found an excitatory effect on elevator motoneurons if stimulated just preceding their normal time of firing. Thus it would appear that the sensory afferents from the tegula discharge slightly before the bottom of the

wingstroke (Neumann 1985) and provide a short-latency excitation to elevator motoneurons which promotes the onset of the upstroke.

3. Campaniform Sensilla. Located on the proximal wing veins of each wing are a group of campaniform sensilla which signal the load experienced by the wing during flight (Gettrup 1966). Their discharge begins slightly before the top of the wingstroke and continues throughout the downstroke, with the maximum activity occurring during the first part of the downstroke (Gettrup 1966). The campaniform sensilla are very important for regulating twisting of the forewings (Gettrup and Wilson 1964, Gettrup 1966, Heukamp 1984). If the body angle of a flying locust in a wind tunnel is changed, the animal changes the twist of its forewings during flight to maintain a constant lift (Gettrup and Wilson 1964). Intact hindwing campaniform sensilla are necessary for this reflex (Gettrup 1966). Intact forewing campaniform sensilla are necessary for free flying locusts to maintain stability (Gettrup 1966). Heukamp (1984) has found that electrical stimulation of the campaniform sensilla elicits reflex firing in the pleuroalar muscle 85, which is important in controlling wing twisting (Pfau 1978a). These receptors also seem to be important in controlling the rhythmic movements of the wings. Stimulation of the campaniform sensilla in quiescent animals, either by bending the wing (Wendler 1978) or electrical stimulation (Horsmann and Wendler 1985) elicits reflex firing of the depressor muscles of all four wings. Recently, Horsmann and Wendler (1985) have reported that electrical stimulation of the campaniform sensilla is a very potent resetting stimulus for the flight rhythm.

4. The Chordotonal Organ. This receptor lies very close to the wing stretch receptors within the wing hinge (see figure 3). It sends approximately 20 afferent axons to the CNS, via nerve $1d_2$ (Gettrup 1962). No recordings of its discharge during flight have been published, except as were incidentally seen by Gettrup (1966), while recording stretch receptor activity. He described both phasic and non-phasic units active during flight. The only proposed function for the chordotonal organ is that of monitoring the degree of wing twist (Pfau 1978b). However, this was based on an anatomical investigation and no

physiological evidence has been brought forward to either support or contradict this hypothesis.

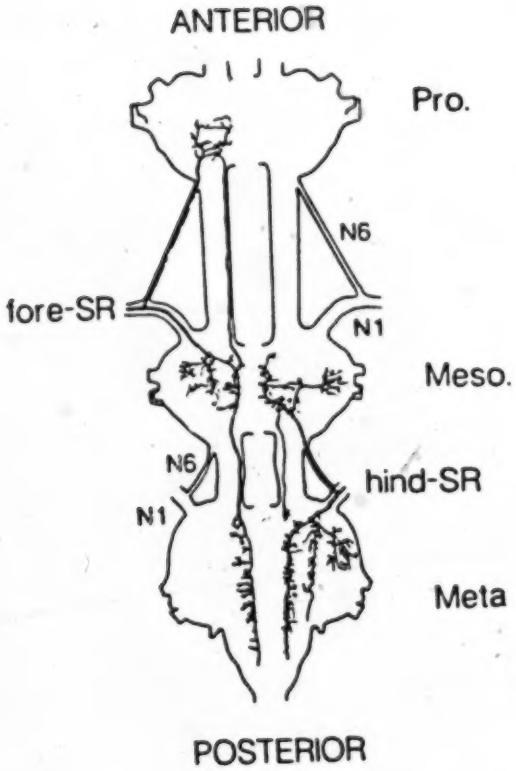
5. The Wing Stretch Receptors

The wing stretch receptors (SRs) are unicellular multipolar receptors located at the hinge of each wing, there being one SR for each wing (Gettrup, 1962). The forewing SR originates on the lateral edge of the mesophragma and inserts behind the subalar sclerite below the posterior part of the wing hinge (Figure 3b, Gettrup 1962). The hindwing SR differs in its origin, arising from the metathoracic postnotum rather than from the phragma.

The SRs lie very close to the chordotonal organ and both send their afferent axons in nerve Id_2, the SR axon being the largest (ca. 12 μm) (Altman and Tyrer, 1977). In common with the other wing afferents, the SR axon enters the thoracic ganglia via n1 but the SR differs because in addition it branches peripherally to send an axon to the next anterior ganglion via the sixth nerve of that ganglion (Burrows 1975, Altman and Tyrer 1977). The central arborizations of the forewing and hindwing SRs are shown in figure 4. The forewing SR projects to all three thoracic ganglia, whereas the hindwing SR projects only to the mesothoracic and metathoracic ganglia (Altman and Tyrer 1977). Each afferent branches extensively in the dorsal neuropil of the ganglia, but these branches are confined to the ipsilateral hemiganglion (Burrows 1975, Altman and Tyrer 1977).

The properties of the wing SRs have been examined by recording their discharge in tethered flying animals (Wilson, 1961; Wilson and Gettrup, 1963; Möhl, 1979, 1985b), in response to mechanically imposed wing movements (Pabst 1965, Wendler 1977, 1983, Wendler and Heukamp 1983) and to direct stretch of the receptor itself (Gettrup 1963). Wilson and Gettrup (1963) recorded the discharge of the forewing and hindwing SRs in tethered, flying *Schistocerca gregaria*. The SRs were found to fire briefly at the top of the wing upstroke. Usually, the SRs discharged only one or two spikes per cycle, although this number increased as the flight frequency dropped (Wilson and Gettrup 1963). The

Figure 4. Arborizations of the forewing and hindwing SR afferents in the thoracic ganglia. The projection of one forewing SR is shown on the left and of one hindwing SR on the right. The forewing SR afferent branches peripherally, projecting into the prothoracic ganglion via pro.n6 and the mesothoracic ganglion via meso.n1. The afferent continues into the metathoracic ganglion via the ipsilateral mesothoracic-metathoracic connective. A fine branch also projects from the prothoracic ganglion into the mesothoracic ganglion. The hindwing SR afferent enters the mesothoracic ganglion via meso.n6 and the metathoracic ganglion via meta.n1. A fine branch descends from the mesothoracic ganglion (from Altman and Tyrer 1977).


ANTERIOR
Pro.
N6
fore-SR
N1
Meso.
N6
hind-SR
N1
Meta
POSTERIOR

maximum burst frequency observed was approximately 200 Hz. A similar relationship has been found in response to imposed sinusoidal wing movements in *Locusta migratoria* (Pabst 1965, Wendler 1983). The recordings of Möhl (1979, 1985b) in intact tethered flying *Locusta* differ significantly. At the top of the wingstroke, the SRs were observed to discharge in high frequency bursts of approximately 500 Hz containing an average 5 spikes per burst, although this could vary from 2-16 spikes. He found no consistent correlation between the wingbeat frequency and the number of potentials in each burst (Möhl 1985b). The differences between Möhl's (1979, 1985b) results and those seen with artificially imposed wing movements (Pabst 1965, Wendler 1977, 1983) appear to lie in the inadequacy of sinusoidal up and down movements to mimic the actual wing movements of flying animals. In particular, during flight the wings also pronate at the top of the upstroke (Jensen 1956). The SRs insert at the posterior part of the wing hinge (Gettrup 1962) and so are also stretched when the wing pronates (Pfau 1978b, Wendler and Heukamp 1983).

The reason for the discrepancy between Möhl's (1979, 1985b) recordings and those of Wilson and Gettrup (1963) is less clear. The latter used more highly dissected preparations and this may have depressed the nervous system somewhat or had an influence on the normal wing-hinge mechanics.

In response to sinuosidal stretching, either by direct stretch (Gettrup 1963) or imposed wing movements (Wendler 1977, 1983), individual SR spikes occur at a particular phase in the movement cycle, dependent on the frequency of stimulation. The higher the frequency of movement the later the first spike occurs with respect to the movement (Gettrup 1963). Wendler and Heukamp (1983) investigated the ability of the forewing SRs to respond to small perturbations of the wing movement. A wing was moved sinusoidally with a similar amplitude and frequency to natural wing movements. These workers then superimposed small perturbations onto these movements. They found that a disturbance of 1% of normal amplitude could produce a phase-shift of as much as 20 degrees, with larger

disturbances producing larger phase-shifts and an increased number of spikes per burst. The higher the maximum wing elevation, the earlier the spike occurred. However, similar phase-shifts were also observed when perturbations in wing twisting were superimposed on the regular sinusoidal movement. Thus, based on the phase of the SR burst, the CNS would not appear able to distinguish between these two conditions.

These experiments show the SRs to be very sensitive to small changes in an artificial wing movement. Möhl (1985b) has extended these observations to natural wing movements. He monitored the up and down movements of one forewing in tethered flying *Locusta* while recording the forewing SR of the same wing. The activity of the SRs was found to accurately reflect events at the top of the wing upstroke. A higher instantaneous frequency of the SR burst was related to a higher amplitude of the upstroke. The number of spikes in each burst for the same amplitude movement reflected the time which it takes for the wing to move through its upper turning point. That is, if the depressors are late in firing, then the wing stays at its top position longer and the duration of the SR burst is prolonged.

Influences of the SRs on the Flight Rhythm

Wilson and Gettrup (1963) lesioned the SRs peripherally in *Schistocerca*. They found that lesioning 2,3, or 4 SRs caused a 10%, 25% and 50% drop, respectively, in the flight frequency. The frequency seen when all four SRs were lesioned was similar to that of completely deafferented preparations. Thus, the SRs appear necessary to obtain the intact flight frequency. The lesion experiments indicated that the flight frequency decreased irrespective of which particular combination of SRs were lesioned, hence they concluded all have equivalent effects on the flight rhythm. It should be noted, however, that these lesions also included the chordotonal organ.

These workers, then performed the converse experiment. They took deafferented preparations and stimulated two SR nerves (one forewing SR and the ipsilateral hindwing SR). Stimulus trains were applied at a constant rate, independent of the flight frequency.

They found that this stimulation increased the flight frequency slowly, reaching a maximum with a time constant of 2 seconds (ca. 25 cycles). The greatest increase in frequency was of the order of 30%. A surprising result was that the flight motor output exhibited no phase-preference with respect to the stimulus. Apparently, the SRs merely increased the excitability of the flight oscillator but the information carried by the SRs as to the details of each wingstroke was not used. These experiments were repeated in a later study (Wilson and Wyman 1965) and the same results obtained. This latter work further noted that in a few animals a strong phase-preference was obtained but in these cases the motor output would start to become arrhythmic.

These findings stood until Wendler (1974) performed experiments to show that sensory input could alter the cycle-by-cycle timing of the flight rhythm. In these experiments animals were tethered and flown in front of a wind tunnel. Via a penmotor, he then forcibly moved one forewing at a slightly different frequency from the other three wings. Within one or two cycles, the non-driven wings adopted the frequency of the driven wing. This had to be interpreted as a rapid, phase-dependent influence of sensory receptors on the flight rhythm. A similar demonstration of cycle-by-cycle influences was found by Möhl and Nachtigall (1978) who electrically stimulated individual muscles during tethered flight. A small resetting of the flight rhythm was evident. However, the receptors responsible for the results seen in either of these experiments remained unknown. Recent results using intact animals show that two proprioceptive groups can reset the flight rhythm. One of these is the wind hairs on the head of the locust (Bacon and Möhl 1983, Horsmann et al 1983) and the other is the wing campaniform sensilla (Horsmann and Wendler 1985). Whether these two groups are responsible for the results of Wendler (1974) is not known.

These studies indicated that the phase-independent influences of the SRs could not be extrapolated to include all proprioceptors. Indeed, based on the above studies, one may doubt that the SRs themselves do not have some phasic influences on the flight motor.

Burrows (1975) examined the connections the SRs made with flight motoneurons in *Schistocerca gregaria*. He found presumed monosynaptic excitatory postsynaptic potentials (EPSPs) in depressor motoneurons and small, presumably disynaptic, inhibitory postsynaptic potentials (IPSPs) in elevator motoneurons. By tonically depolarizing one depressor motoneuron (the forewing first basalar), he could obtain rhythmic firing of the motoneuron by superimposing trains of stimulation to the SR nerve. From these results, Burrows suggested that the SRs formed part of a negative feedback loop. The elevator motoneurons excite the SRs which in turn inhibit the elevators and promote the onset of the depressor firing. The connections of the SRs to motoneurons suggested that the timing information in the SR discharge could be used to control the time of firing of the depressor muscles.

The state of knowledge at the start of this project may be summarized as follows. The SRs can provide detailed information concerning the wingstroke. This is encoded in the burst frequency, the number of spikes and the timing of the burst within the wingbeat cycle. However, this information appears not to be used by the central oscillator to modify its timing. Rather, the SRs appear to merely increase the general excitability of the central flight oscillator. Nevertheless, the connections of the SRs to flight motoneurons suggest that they may be able to influence the time at which motoneurons fire. Recent experiments in tethered flying animals (Wendler 1974, Möhl and Nachtigall 1978) showed that wing proprioceptors can have rapid cycle-by-cycle influences on the timing of the flight rhythm. The receptors responsible are unknown but the possibility that the SRs are involved cannot be ruled out.

In this project, the influences of the SRs on the flight rhythm were re-investigated. These experiments showed that the forewing SRs reset the flight rhythm generator in a phase-dependent manner. They increase the flight frequency and markedly prolong the duration of flight sequences in deafferented preparations. These experiments are described in the second chapter. In the third chapter, I show that these effects can also

be produced in deafferented preparations and suggest reasons for the failure of previous workers to see phase-dependent effects. The fourth chapter describes thoracic interneurons and motoneurons which receive short-latency excitatory post-synaptic potentials from the wing SRs. A number of these were further examined for their response to SR stimulation during expression of the flight rhythm. It was found that this stimulation could cause major alterations in the activity of some of these neurons. In the fifth chapter, a group of neurons are described with properties consistent with their being involved in the initiation and maintenance of flight. The influence of SR stimulation on the activity of these neurons was examined to investigate their role in the increase in frequency and prolongation of flight sequences elicited by SR stimulation. In the final chapter, the major results of the thesis are discussed and compared with other systems.

II. INFLUENCE OF SRs ON THE SEMI-DEAFFERENTED FLIGHT RHYTHM.

A. INTRODUCTION.

The most noticeable feature of the flight rhythm in deafferented locusts is that the frequency of the rhythm is only 50% of that seen in intact locusts (Wilson 1961, Kutsch 1974). By lesioning specific groups of proprioceptors Wilson and Gettrup (1963) showed that two wing stretch receptors must be left intact in order to observe the normal flight frequency. Their studies on the relationship between wing movements and SR discharge showed that it could provide timing information to the central oscillator (Gettrup 1963, Wilson and Gettrup 1963). However, when these workers stimulated the afferents from two stretch receptors in deafferented preparations (Wilson and Gettrup 1963, Wilson and Wyman 1965) they found that the flight frequency increased slowly over 2 - 4 seconds but that this effect did not depend on the timing of the stimulation in the flight cycle. They concluded that the timing information carried by the SRs was not used and that the SRs elevated the flight frequency by increasing the general excitability of the central nervous system. This has given rise to the concept that a phasic proprioceptor can have purely phase-independent effects on a central rhythm. Yet the evidence for this concept is almost solely derived from the work on the locust flight system. Two more recent studies on locust flight (Wendler 1974, Möhl and Nachtigall 1978) have both demonstrated that wing proprioceptors can reset the flight rhythm in tethered flying locusts. The actual proprioceptors responsible were not identified. One possibility is that the SRs were involved.

The experiments described in this chapter were carried out to re-examine whether the SRs have a phase-dependent influence on the timing of the flight rhythm. They took advantage of more recent knowledge of the peripheral anatomy of the forewing SR afferents (Burrows 1975) which allowed selective electrical stimulation of the forewing SR afferents during flight activity. These experiments found that stimulating the forewing SRs caused a dramatic increase in the flight frequency and markedly prolonged the duration of flight sequences. These effects

were dependent on the time in the flight cycle at which the stimulation was given. Single stimulus trains applied to both forewing SRs reset the flight rhythm and presentation of stimulus trains at a constant rate could entrain the rhythm. These experiments demonstrated a strong phase-dependent influence of the SRs on the timing of the flight rhythm generator.

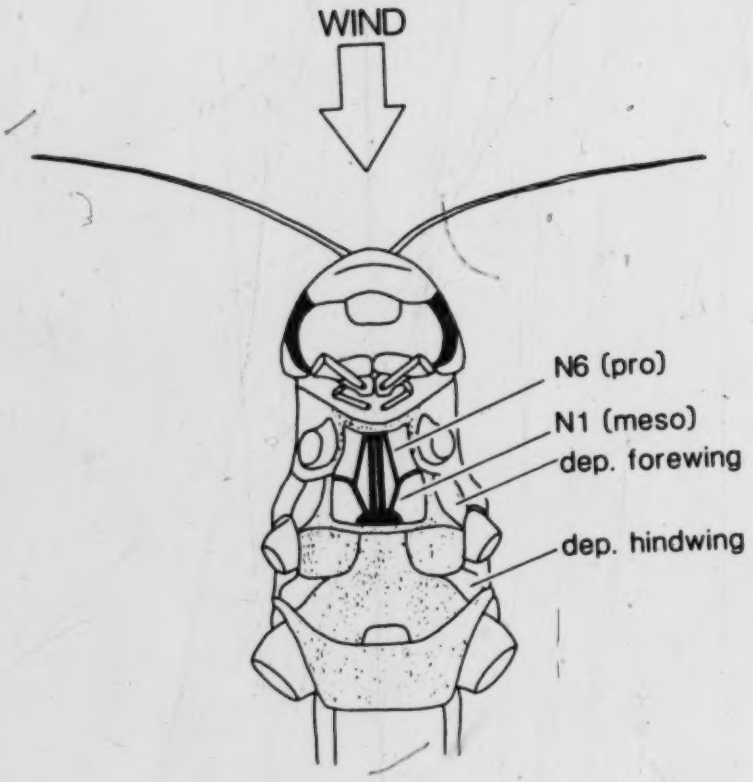
B. MATERIALS AND METHODS.

Experiments were performed on mature adult male and female *Locusta migratoria* obtained from a long established colony at the University of Alberta. No differences were found related to sex. All experiments were carried out at room temperature (22° - 24°C).

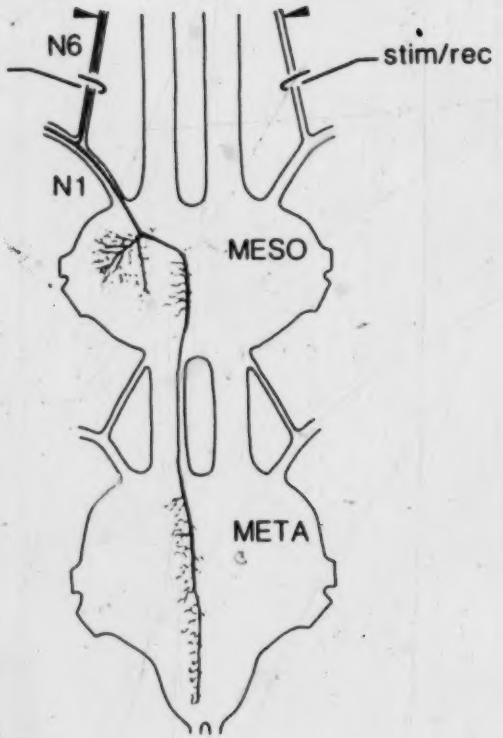
I refer to the preparation used in the experiments described in this chapter as the "semi-deafferented" preparation. Following amputation of the legs and wings, animals were mounted ventral side up on a corkboard. They were restrained by 2 pins placed through the lateral pronotum. The ventral cuticle overlying the mesothoracic ganglion was removed, exposing the two sixth nerves of the prothoracic ganglion and their junction with the first nerves of the mesothoracic ganglion. To allow unrestricted movement of the wing stumps, the animals were raised slightly above the corkboard. Flight activity was elicited by directing a wind stream to the head of the animal from a 12mm tube placed 8cm in front of the animal. The wind source was a compressed air cylinder connected to the tube via a pneumatic relay. Wind velocities were kept in the range of 1 - 3 m/s. The flight motor output was usually recorded from the first basalar muscle (depressor) of the hindwing via a 100μm copper wire, insulated except for the tip, placed in the muscle at its insertion on the ventral cuticle. In some cases the forewing first basalar muscle was recorded. The preparation is illustrated in Figure 5.

The f-SRs (forewing SRs) were stimulated at their peripheral branch in the 6th nerve of the prothoracic ganglion. Other sensory afferents from the forewing do not possess a branch in this nerve (Tyrer and Altman 1974, Altman and Tyrer 1977). Hence, by stimulating pro.n6 one should be able to selectively stimulate the f-SR afferents. This procedure was employed by Burrows (1975). To ensure that stimulating pro.n6 excited only the f-SR afferent and not other

Figure 5. Schematic illustration of the experimental preparation. A: Locusts were placed ventral side up on a corkboard after removing the legs and wings. A piece of central cuticle was removed to expose the sixth prothoracic and first mesthoracic nerves. The depressor EMGs (first basalar) were recorded by wires inserted just under the ventral cuticle at the points indicated. Flight activity was elicited by a windstream directed to the head. B: Site of stimulation in relation to the forewing SR afferent. Monopolar stimulating electrodes were placed on each pro.n6 with that nerve cut or crushed proximally to the prothoracic ganglion (arrows). The antidromic spike invaded into the main branch of the afferent which then projected into the meso- and meta-thoracic ganglia.


WIND
N6 (pro)
N1 (meso)
dep. forewing
dep. hindwing

A


N6
stim/rec
N1
MESO
META

B

wing afferents, recordings were made in several animals from meso.n1 where it entered the mesothoracic ganglion and pro.n6 was stimulated. The results of this experiment are illustrated in Figure 6. The spontaneously occurring f-SR spike is seen in both pro.n6 and meso.n1 in Figure 6b. Stimulating pro.n6 elicited a single unit in meso.n1 of the same amplitude and time course as the spontaneously occurring f-SR spike (Fig.6c). No other units were apparent at voltages up to three times threshold (ca. 1.0v).

Because motoneuron axons (to the dorsal longitudinal muscle) arising from the prothoracic ganglion also travel in this nerve, the nerves were crushed or cut close to the prothoracic ganglion to prevent antidromic stimulation of the dorsal longitudinal motoneurons. To prevent excitation of the dorsal longitudinal muscles via their motoneuron axons and also to prevent the natural f-SR discharge from reaching the CNS, the first nerves of the mesothoracic ganglion were cut peripherally to their junction with the prothoracic sixth nerves. Hence the preparation was deprived of sensory feedback from the forewings.

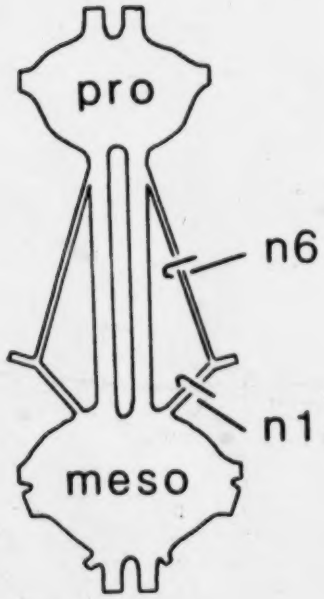
Stimulation Procedures.

Monopolar stimulating electrodes (75μm Ag or Pt wire) were placed on each pro.n6 with the indifferent of each electrode pair placed in the haemolymph lateral to the prothoracic ganglion. Each electrode pair was connected to a separate stimulator which was used to set the stimulus train parameters. A third stimulator was used to trigger these two stimulators. Stimuli were delivered to both nerves as short trains of pulses, each being 0.1 msec in duration. Train durations were varied over a range of 10 to 35 msec but were generally 30msec duration at frequencies of 200 - 400 Hz.

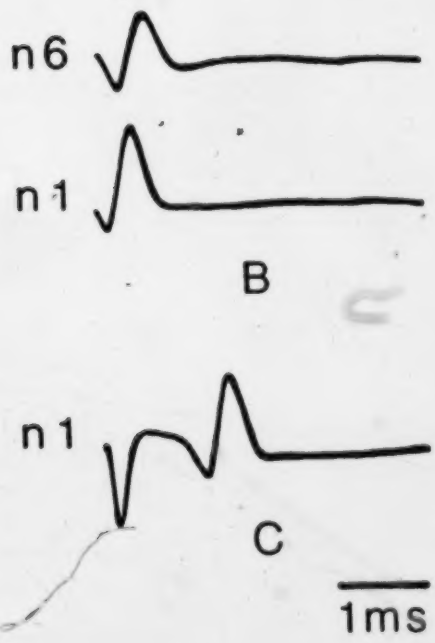
The major purpose of the experiments described in this chapter was to examine whether the effects of the forewing SRs on the flight rhythm were dependent on the time in the flight cycle at which the SRs were stimulated. Three different types of experiment were performed to determine that the SRs exerted a phase-dependent influence on the flight rhythm generator.

(i) The output from the flight CPG (usually the hindwing depressor spike) was used to trigger

Figure 6. Selective stimulation of the forewing stretch receptor afferent. A: Electrodes were placed on pro.n6 and meso.n1. B: The spontaneous SR spike was seen in both electrodes, occurring in n1 slightly before it appeared in n6. C: A single stimulus pulse applied to pro.n6 evoked a single unit recorded at meso.n1. This had the same amplitude and time course as the spontaneously occurring SR spike seen in B.



A



the stimulators on every cycle. The time between the muscle spike and the triggering of the stimulators could be varied. Hence comparisons could be made among trials with different delays between the depressor spike and the onset of the stimulus train.

(ii) Single stimulus trains were applied to the forewing SRs to occur every 6 - 8 cycles of the flight rhythm. These stimuli would occur at many different times in the flight cycle. The effects of each stimulus train on the duration of subsequent flight cycle periods were calculated as a function of the time in the cycle at which that stimulus fell.

(iii) Stimulus trains were delivered at a constant rate independently of the flight rhythm. These experiments tested whether repetitive SR stimulation could entrain the flight rhythm. That is, whether the flight rhythm would adopt the frequency of the SR stimulation.

Initially, the EMG and stimulus pulse were reproduced on a chart pen recorder. On-line plots of the instantaneous oscillator frequency versus time were displayed on a storage oscilloscope and photographed. In later experiments, the depressor EMG and stimulus trigger pulses were recorded on magnetic tape. Interval measurements were carried out using a digital computer. An on-line plot of the oscillator frequency was obtained using an instantaneous frequency meter (Neurolog NL255) and the output displayed on a chart pen recorder.

The phase-preference obtained during entrainment experiments was calculated as follows. The stimulus period was taken as the time between consecutive stimulator trigger pulses (corresponding to the beginning of a stimulus train). Phase was calculated by dividing the latency of the first depressor spike following the last stimulus trigger pulse divided by the stimulus period. Hence, if the depressor spike exactly coincided with the beginning of the stimulus, its phase in the cycle = 0. Values were converted to corresponding values in the stimulus half cycle. Depressor spikes occurring in the first half of the stimulus period had phase values in the range (0.0 - 0.5). Spikes falling in the latter half of the stimulus period fell in the phase range (-0.5 - 0.0).

C. RESULTS.

Characteristics of Preparation.

The following describes the flight characteristics of the semi-deafferented preparations before cutting the forewing sensory nerve. A constant windstream directed to the head elicited rapid rhythmic movements of the wing stumps. Elevator and depressor muscles fired alternately and the hindwing to forewing intersegmental delay was still apparent (see Fig.9). This motor pattern resembled that seen in intact flying locusts (Wilson and Weis-Fogh 1962, Möhl 1985a). Typically, the initial frequency fell in the range of 16 - 20 Hz but gradually declined over time until the rhythm stopped at levels below 8 Hz. Sequences usually lasted 4 - 10 seconds, although there were also exceptions where the rhythm continued for over a minute. Increasing the wind velocity caused a slight elevation in the average frequency and longer flight sequences but these effects were not marked (Fig.7). Cutting the forewing sensory nerve caused lowering of the rhythm frequency and a further shortening of the duration of flight sequences (Fig.8).

In order to determine appropriate timing with which to stimulate the forewing SR nerves, the intact f-SR discharge was recorded from pro.n6 in several animals (the forewing sensory nerve was left intact). An example of the recorded activity is shown in Fig.9. The SR spikes occurred in short high frequency bursts close to the time of depressor activity. The intraburst frequency generally was 300 - 400/s, with 3 - 6 spikes occurring in each burst. These bursts resembled those recorded by Möhl (1979, 1985b) in intact, tethered flying locusts.

Stimulation of Forewing SRs.

Initially, the effects of the forewing SRs on the flight rhythm were examined using triggered stimulation ($n>20$). That is, the stimulus trains were triggered to occur on every cycle with a set latency following the depressor EMG. Stimulating both f-SR nerves at 0msec latency during a flight sequence had two dramatic effects on the flight rhythm. The first was a rapid increase in flight frequency (Fig.10). The final frequency reached was within the range of

Figure 7. Characteristics of flight activity in locusts, fixed ventral side up, wings and legs removed. Plot of wingbeat frequency (average of 4 cycles every 0.25 sec) as a function of time from the onset of flight. A continuous windstream was directed to the head at three different velocities: 1m/s (triangles), 2m/s (closed circles), 2.5m/s (open circles). Data from three separate trials in one animal.

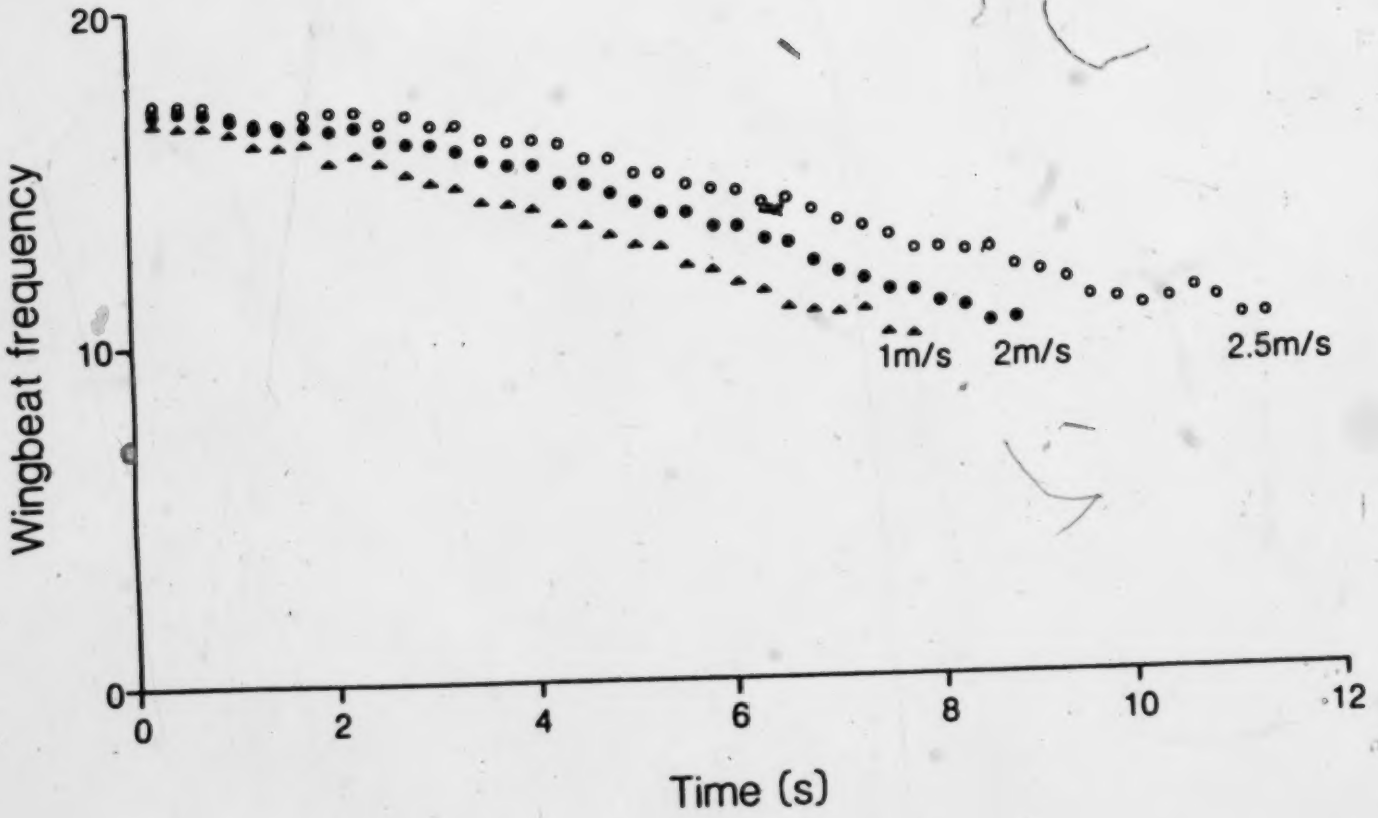

Wingbeat frequency
20
10
0
0
2
4
6
8
10
12
Time (s)
1m/s
2m/s
2.5m/s

Figure 8. Effect on flight activity of bilaterally cutting the forewing sensory nerves (meso.n1) distally to their junction with the prothoracic nerves 6. Plot shows the instantaneous wingbeat frequency as a function of time following the onset of a continuous wind stimulus (2m/s) to the head. Cutting the meso.n1s caused the flight frequency to decrease more rapidly and also shortened the duration of the flight sequence.

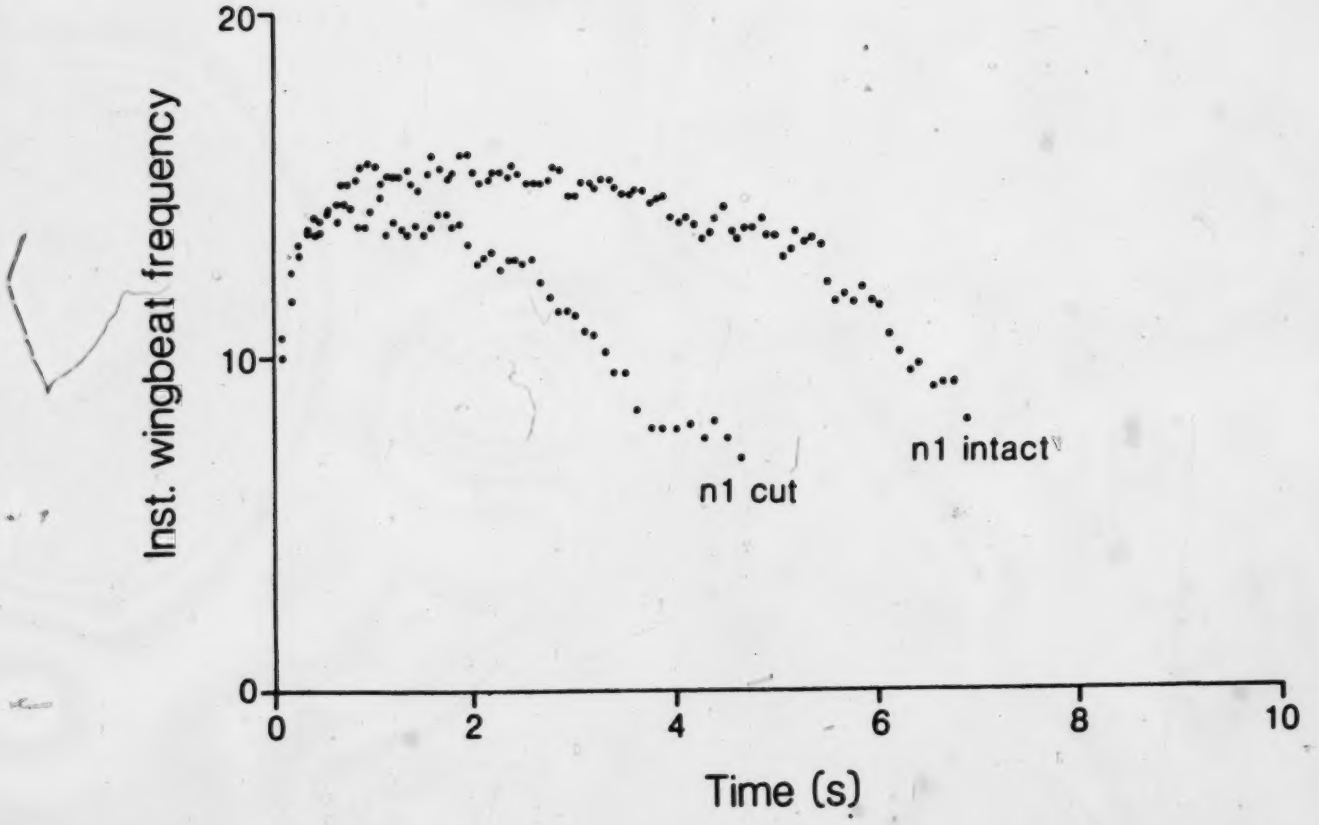

Inst. wingbeat frequency
20
10
0
0
2
4
6
8
10
Time (s)
n1 intact
n1 cut

Figure 9. Activity of the forewing stretch receptors in relation to the flight motor output. A and B: Two series of flight cycles recorded in the same animal, B taken some time later than A. Upper and middle records: the depressor EMGs from the first basalar of the forewing and hindwing respectively. The hindwing to forewing intersegmental delay was still apparent. Lower record: Discharge of the forewing SRs recorded at meso.n1 near its root at the mesothoracic ganglion. Bursts contained 3 ~ 7 spikes usually at frequencies of 300 Hz or more. The bursts slightly preceded the forewing depressor, occurring close to that of the hindwing depressor. m97: forewing first basalar. m127: hindwing first basalar.

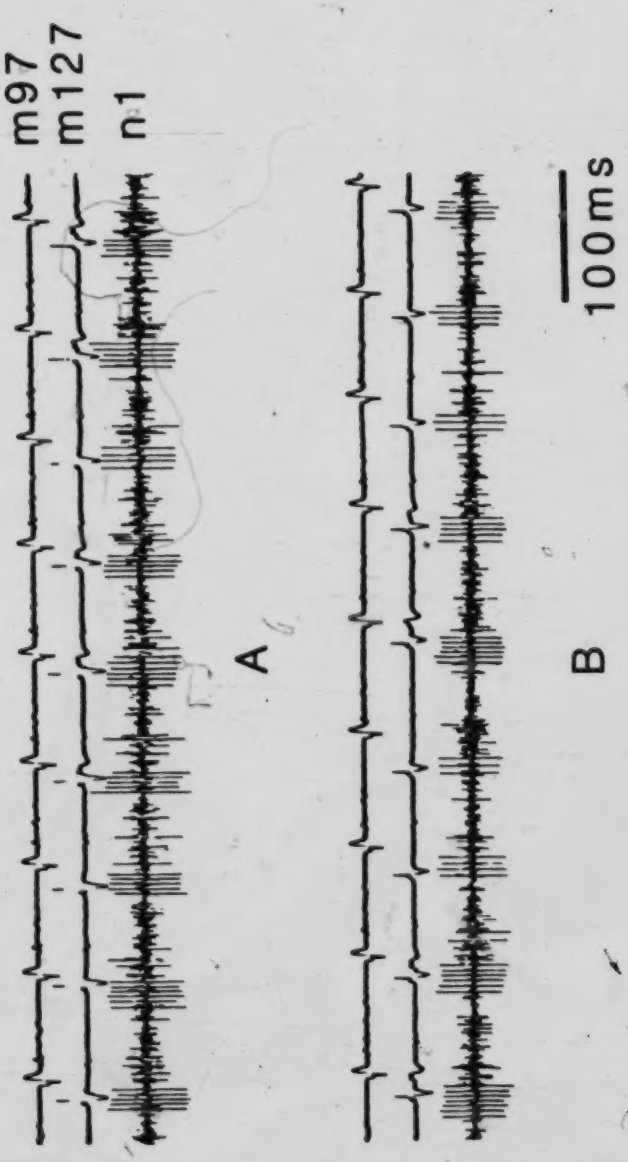
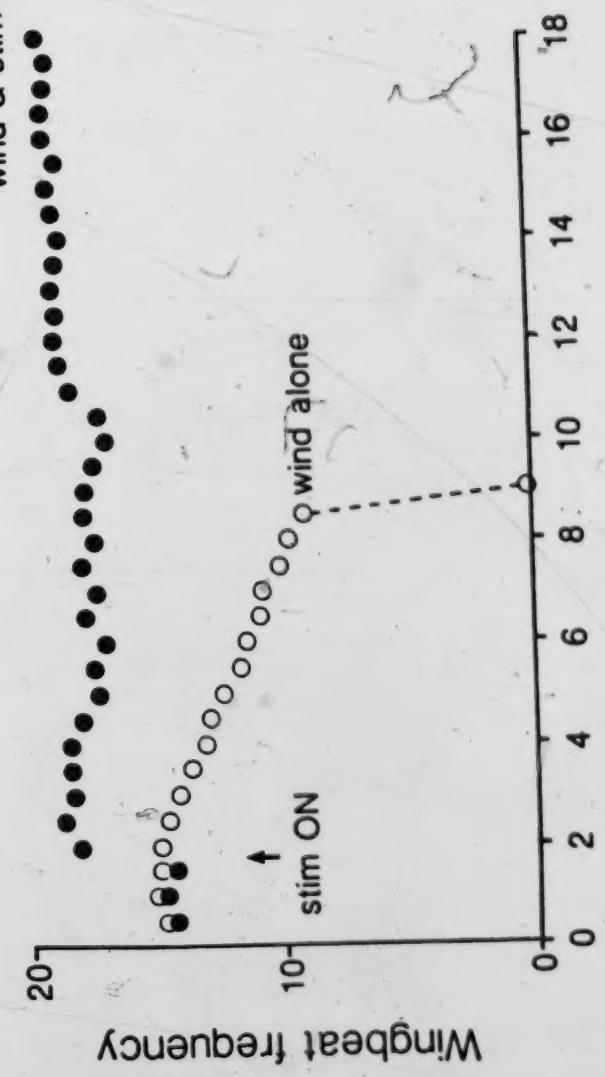

m97
m127
n1
A
B
100ms

Figure 10. Increase in flight frequency and prolongation of flight sequence with bilateral forewing stretch receptor stimulation. Wingbeat frequency (average of 3 cycles every 0.5 sec) versus time from two representative trials for two conditions. In the trial represented by the open circles, only the wind stimulus was given. In the other trial (filled circles), the wind stimulus was also given but two seconds after the commencement of flight activity, the forewing stretch receptors were stimulated to occur on every cycle, immediately following the hindwing depressor spike. There was a marked elevation in the flight frequency and the duration of the sequence was also prolonged. The wind stimulus was applied continuously throughout both trials.


wind & stim
wind alone
stim ON
Wingbeat frequency
Time (s)
20
10
0
0
2
4
6
8
10
12
14
16
18

16 - 20Hz, typical values seen in tethered flying animals. When the stimulation was stopped, the frequency declined quickly followed by a slowing of the rhythm which paralleled the frequency profile seen when no stimulation accompanied the flight sequence (not shown). The second conspicuous effect of the stimulation was that flight sequences were considerably longer than those trials when no stimulation was presented (Fig.10). In a few animals, flight sequences would continue for several minutes with SR stimulation whereas the wind alone trials lasted only 4 - 10 seconds.

Although Wilson et al also reported that SR stimulation increased the flight frequency, an essential difference seen in the current study was that the effects occurred very rapidly. This can be seen in Figure 11a. Here the time course of the decrease in cycle period is shown for the 8 periods following the introduction of the stimulus. The period decreased on the first cycle of stimulus presentation and the maximum shortening was usually reached in 6 - 10 cycles. The converse experiment is illustrated in Figure 11b. Here, the flight frequency had previously reached a steady level with f-SR stimulation. The stimulators were then turned off. This resulted in a lengthening of the period on each subsequent cycle, beginning on the first cycle after cessation of stimulation.

The next experiments examined whether this effect on flight frequency and duration depended on the timing of the stimulation within the flight cycle. Here, the latency between the depressor spike and the stimulus was varied. A clear phase-dependence in the effects of SR stimulation was found (Fig.12). Stimulation timed to occur immediately after the hindwing depressor spike caused a rapid increase in frequency and prolonged the duration of the flight sequence. On the other hand, stimuli timed to occur midway through the cycle (delay=35msec) had no effect on the rhythm. The frequency profile seen for the latter was very similar to that found when no SR stimulation accompanied the wind stream (not shown).

To further investigate this phase-dependence, the effects on the flight rhythm of single stimulus trains were examined. The aim of these experiments was to stimulate the forewing SRs every 6 - 8 cycles with the train falling at many different times in the flight cycle. The effects

Figure 11. Time course of changes in wing beat period in response to turning on and turning off SR stimulation. A: Time course of decrease in wingbeat period following commencement of bilateral forewing stretch receptor stimulation. Flight activity was initiated by a continuous wind stimulus directed to the head. After 1.5 seconds, the forewing stretch receptors were stimulated to occur on every flight cycle, immediately following the hindwing depressor spike. Data from 10 trials in one animal have been averaged and the period normalized to the period of the interval immediately preceding the onset of f-SR stimulation. For each interval the mean (filled circles) and standard deviation (vertical bars) are indicated. The period progressively decreases for 6 cycles following the onset of stimulation and the first stimulus decreases the flight period. B : Time course of the increase in flight period following cessation of f-SR stimulation. Data from the same animal as in A. In this case the flight frequency had reached a steady maximum with SR stimulation. The stimulators were turned off. Each period was normalized with respect to the period immediately preceding turning the stimulation off. Data were averaged from 9 trials (in one case, the flight sequence stopped in less than 8 cycles after cessation of stimulation - this sequence was not included in these data). The period increases rapidly after ceasing bilateral f-SR stimulation.

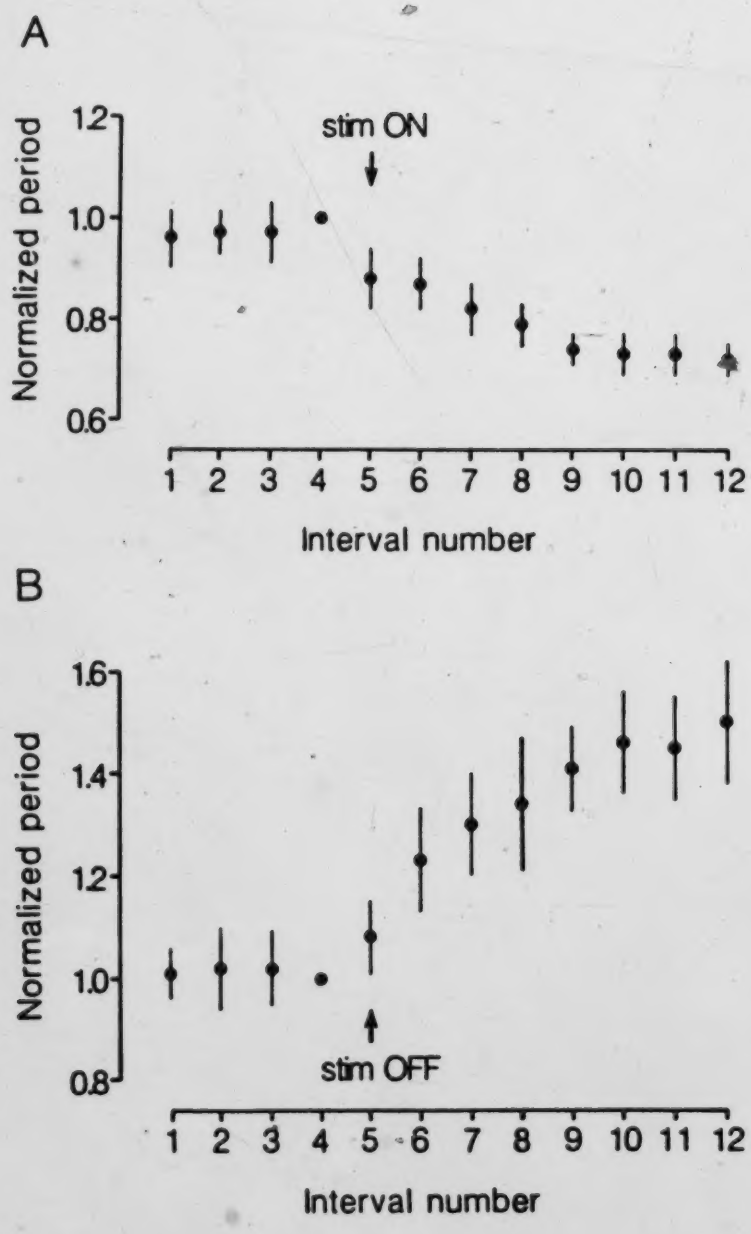
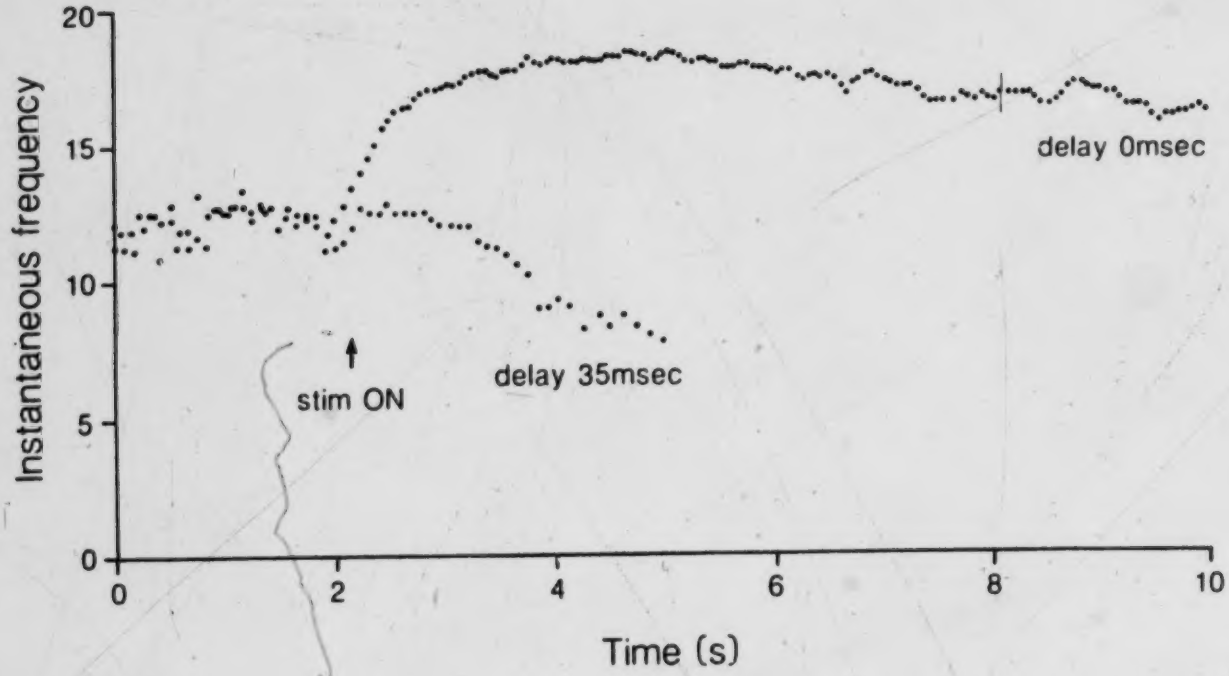

A
Normalized period
1.2
1.0
0.8
0.6
stim ON
1 2 3 4 5 6 7 8 9 10 11 12
Interval number
B
Normalized period
1.6
1.4
1.2
1.0
0.8
stim OFF
1 2 3 4 5 6 7 8 9 10 11 12
Interval number

Figure 12. Elevation in wingbeat frequency and increased duration of flight sequences depends on the timing of SR stimulation in the flight cycle. Instantaneous frequency versus time for two trials from the same animal are superimposed. In both cases, a constant wind stimulus was applied to the head. Approximately two seconds after the initiation of flight, stimulation was applied to the forewing SRs on every flight cycle. The time between the hindwing depressor spike and each stimulus train was varied. In one trial, stimulation occurred immediately following the depressor spike (delay 0msec). In the other, stimulation commenced 35 msec following the depressor spike. With zero delay, there was a rapid increase in flight frequency and marked prolongation of the flight sequence. These effects did not occur with a 35 msec delay. The profile of flight activity for the latter was very similar to that seen when no SR stimulation accompanied the wind stimulus (not shown).

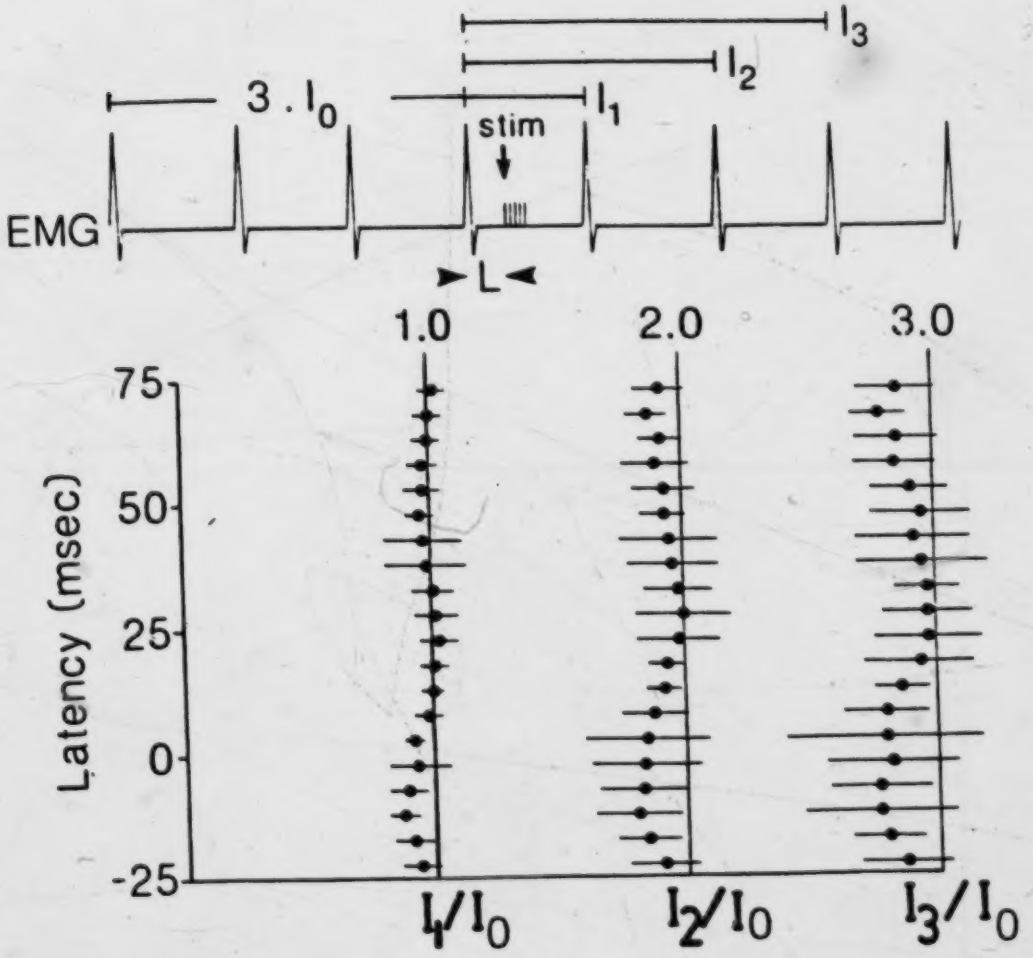

Instantaneous frequency
20
15
10
5
0
0
2
4
6
8
10
Time (s)
delay 0msec
delay 35msec
stim ON

on subsequent, post-stimulus cycles could be examined. Unfortunately, a major limitation to these experiments was obtaining preparations which gave long flight sequences in response to a windstream. As described above, the flight sequences in these preparations were usually of short duration and showed a steady decline in frequency with time. Imposed on this were often considerable cycle to cycle fluctuations in period. They were poorly suited to this type of experiment, particularly regarding obtaining enough points for these curves. Thus, the results presented here were performed on a few animals which flew for long periods of time without forewing SR stimulation. In this respect they were atypical preparations. However, the qualitative results were comparable for the three animals that were tested.

Single stimulus trains were presented at a rate of 1.5/s during the flight sequence. Stimuli fell across all times in the flight cycle. The times at which the following three post-stimulus depressor spikes occurred were normalized with respect to the mean of the three pre-stimulus cycle periods. These animals did show slow changes in average period with time, so points were taken where the average of the 3 pre-stimulus periods fell within 5% of a preselected value (80 msec in Fig.13).

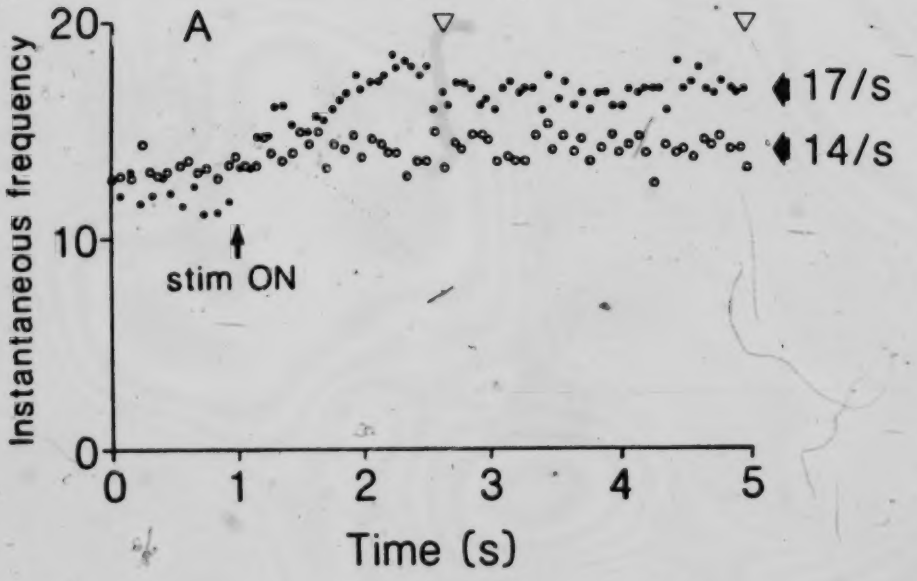
Resetting curves for one animal are shown in Figure 13. The relative time at which the three subsequent depressor spikes occurred is represented on the abscissa. The vertical lines represent the time at which the depressor spike should have occurred had the stimulus had no effect on the rhythm. On the ordinate is the latency of the stimulus with respect to the depressor spike. To preserve continuity in the plot, stimuli falling just before the depressor spike were plotted twice, either as a latency immediately preceding a depressor spike (-25 - 0msec) or at a latency following the depressor. The important qualitative observation was of regions around the depressor phase (0 latency) where the stimulus advanced the timing of the next depressor spike. This advance was not compensated on the next cycle, hence the rhythm was reset. This phase response curve agreed qualitatively with the results seen with triggered stimulation. That is, stimulus trains presented close to the depressor phase decreased the period of the rhythm while stimuli presented in the middle of the cycle had no effect.

Figure 13. Resetting of the flight rhythm by single stimulus trains applied to the forewing SR afferents. Single stimulus trains were applied every 6 - 8 cycles during the flight rhythm. The latency (L) was defined as the time between the onset of the stimulus and the last depressor spike. For cases where the stimulus fell within the 25msec preceding a depressor spike, the latency was given a negative value of -25 to 0 msec. Thus, points falling toward the end of the flight cycle were plotted twice. The times at which the following three depressor spikes occurred were normalized with respect to the average of the three flight cycles immediately preceding the cycle containing the stimulus (I_o). These values were plotted as a function of the latency of the stimulus (ordinate). Each point shows the mean and standard deviation for 5 msec bins of stimulus latency. The vertical bars indicate where the depressor spike should have occurred had the stimulus had no effect on the rhythm. At regions around the depressor phase (latency 0msec), the stimulus advanced the timing of the rhythm and this advance was maintained for subsequent cycles, hence the rhythm was reset. Points for this graph were taken where the mean period was within 5% of 80msecs (n=528).


I_3
I_2
$3 . I_0$
I_1
stim
EMG
L
1.0
2.0
3.0
75
50
25
0
-25
Latency (msec)
I_1/I_0
I_2/I_0
I_3/I_0

The next experiments investigated whether SR stimulation could entrain the flight rhythm. SR stimulus trains were presented at a constant rate independently of the flight rhythm(n=8). It was found that SR stimulus trains presented at a constant rate were strong entraining stimuli. Typically, the rhythm could be entrained over a range of 4 - 6.Hz greater than the prestimulus frequency. For example, an animal with flight frequency of 11 Hz could be entrained to SR trains presented at 17 Hz (Fig.14). Entrainment occurred within 2 seconds of commencing the SR stimulation. The range of frequencies over which entrainment could be obtained was wide if the entrainment frequency was above the intrinsic flight frequency. This indicated the SRs can advance the flight rhythm (Enright 1965, Perkel et al 1964). It was not possible to demonstrate that the SRs could delay the rhythm using the entrainment paradigm. This was because the flight frequency in these preparations continually declined in the absence of stimulation. If the entrainment frequency was set to a lower value than the intrinsic frequency, then the rhythm appeared to slow in a similar manner to that seen without stimulation. Entrainment would occur when the flight frequency declined to the set entrainment frequency. This could take several seconds, depending on how quickly the flight frequency declined in that particular preparation. An example is shown in Figure 15. Stretch receptor stimulus trains were presented at two different rates of approximately 17/s and 12/s. The stimulation at 17/s was above the intrinsic frequency of the flight rhythm. On commencement of the stimulation, the rhythm quickly increased and the flight rhythm adopted the entraining frequency within one second. On the other hand, when stimulus trains were presented at 12/s, the entraining frequency was below the intrinsic frequency of the rhythm. In this case, a longer time was required before entrainment occurred (approximately 3.5 seconds). The slow rate at which entrainment occurred when the intrinsic flight frequency was higher than the entraining frequency suggested that the ablility of the SRs to delay the rhythm was much weaker than their ability to advance it.

Figure 14. Entrainment of the flight rhythm by forewing SR stimulation. A. Instantaneous flight frequency versus time. Flight activity was elicited by a constant windstream to the head. At the time indicated by the arrow, the forewing SRs were stimulated at a constant rate. Two different trials from the same animal are superimposed. In one, the entraining frequency was 17/s (filled circles), in the other the entraining frequency was 14/s (open circles). B. Frequency histograms of the phase in the stimulus cycle at which the depressor spike occurred for these two trials. The portions of the flight sequence analyzed are indicated by the open triangles in A. Note that the depressor spikes fell close to the occurrence of the stimulus (the onset of the stimulus train phase = 0).


A
20
10
0
Instantaneous frequency
17/s
14/s
stim ON
0
1
2
3
4
5
Time (s)

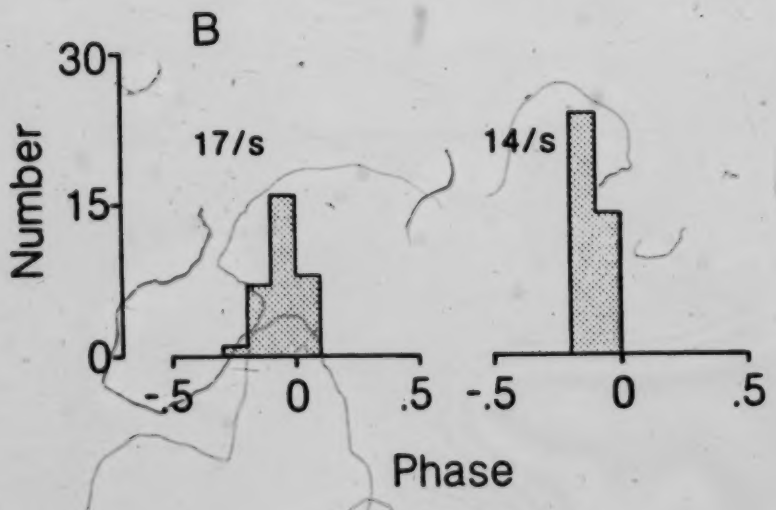
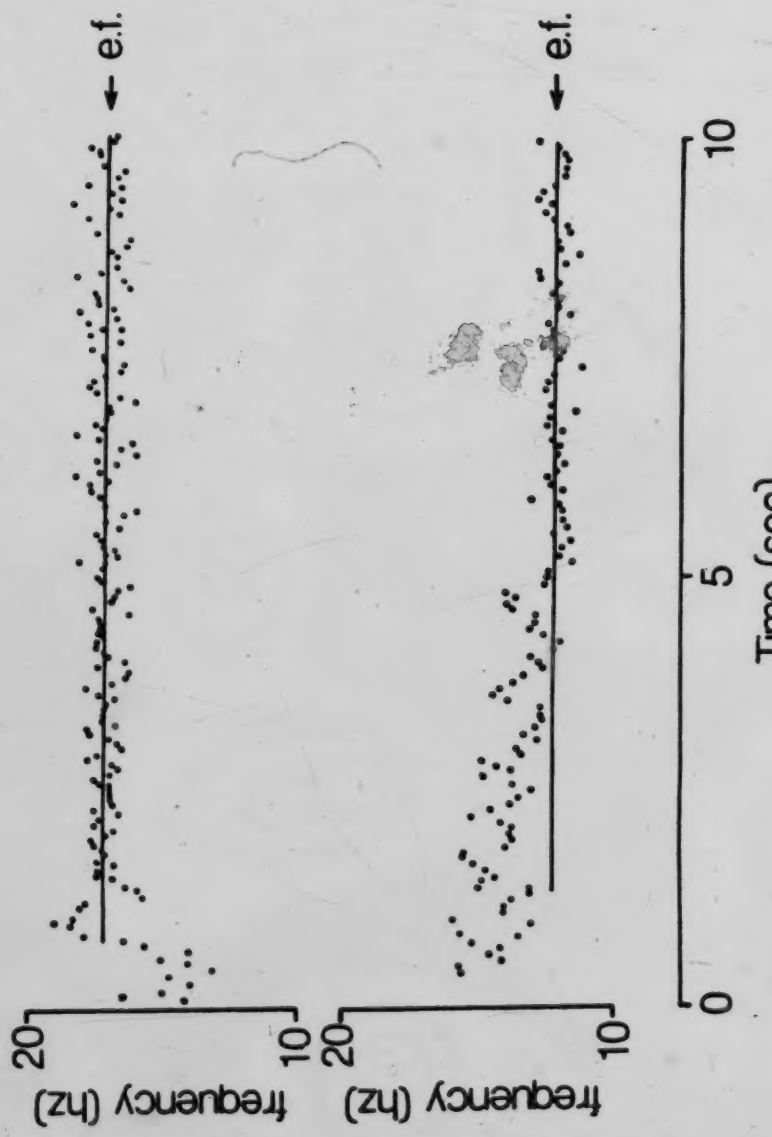

B
30
15
0
Number
17/s
14/s
-.5
0
.5
-.5
0
.5
Phase

Figure 15. SR stimulation preferably entrains the flight rhythm to higher frequencies. Two examples of entrainment of the flight rhythm by bilateral stimulation of the forewing SRs. Both graphs show instantanteous flight frequency versus time for two different trials in the same animal. The frequency of SR stimulation (e.f.= entraining frequency) is indicated by the solid horizontal lines. The beginning of each of these lines indicates the onset of stimulation. Upper graph: SR stimulus trains presented at ca.17/s. This level was higher than the intrinsic frequency of the flight rhythm. The flight frequency increased rapidly following the onset of stimulation and entrainment was established within 1 second. Lower graph: SR stimulus trains were presented at ca.12/s. This level was lower than the initial intrinsic frequency of the flight rhythm. In this case the stimulation was much slower in entraining the flight rhythm, taking over 3 seconds before the entraining frequency and the flight frequency were the same.


e.f.
e.f.
20
10
20
10
frequency (hz)
frequency (hz)
0
5
10
Time (sec)

D. DISCUSSION.

The important result from these experiments was the observation that the SRs had strong phase-dependent influences on the locust flight rhythm. It was proposed that the SRs cause a slow phase-independent increase in the general excitability of the central nervous system (Wilson and Gettrup 1963, Wilson and Wyman 1965). In contrast, these results demonstrate that the SRs exert a rapid, phase-dependent influence which acts on the flight rhythm generator. Stimulating the SRs on every cycle increased the flight frequency and prolonged the duration of flight sequences (Fig.10). The frequency increased on the first cycle of stimulus presentation. Conversely, the cessation of stimulation resulted in a decrease in the flight frequency beginning on the first cycle where the stimulus was not presented (Fig.11). The ability of the SRs to increase the flight frequency and to prolong the duration of flight sequences depended on the SR stimulation being timed to occur close to the time of the depressor spike. Stimulation occurring midway between depressor spikes had no obvious effects on either the frequency or duration of flight sequences (Fig.12). Other experiments have shown that the increase in frequency was due to an influence on the flight rhythm generator. Single stimulus trains occurring close to the depressor spike reset the timing of the flight rhythm and presentation of SR stimulation at a different rate from that of the flight rhythm could entrain the flight rhythm. There was no evidence which suggested phase-independent effects of the SRs on the flight rhythm.

The fact that the SRs can reset the flight rhythm generator shows that they can influence the flight rhythm generator. However, an important point is that, in the intact animal, the SRs will influence the rhythm generator on every cycle. The discharge of the SRs is mechanically linked to the wing movements. Hence, they are active on every cycle, at approximately the time at which the depressor muscles fire (Wilson and Gettrup 1963, Möhl 1985b). The resetting curve (Fig.13) shows that at this time in the flight cycle the SRs advance the flight rhythm. Thus, the SRs should continually act to advance the rhythm on every cycle. This explains why lesioning the SRs causes a decrease in the flight frequency (Wilson 1961,

Wilson and Gettrup 1963, Kutsch 1974).

The SRs meet all the criteria currently used for ascribing a rhythm generator function to a neuron. They can reset the rhythm, they are rhythmically active on every flight cycle (Wilson and Gettrup 1963, Möhl 1985b) and removing the SRs changes the rhythmical motor output (Wilson and Gettrup 1963). Further discussion of this point is left to the final chapter.

III. INFLUENCES OF THE SRs ON THE DEAFFERENTED RHYTHM.

A. INTRODUCTION.

The experiments described in this chapter were concerned with two questions. The first was why did the earlier work of Wilson and colleagues fail to reveal a phase-dependent influence of the SRs on the central flight rhythm?

These workers (Wilson and Gettrup 1963, Wilson and Wyman 1965) used deafferented preparations and found that stimulation of a "lateral" pair of SR's (one forewing SR and the ipsilateral hindwing SR) could not alter the timing of the central rhythm on a cycle-by-cycle basis. Rather, stimulation had a "tonic" excitatory effect on the rhythm, slowly increasing the flight frequency with a time constant of 25 cycles (2 seconds), with no apparent phase preference shown between the stimulus and the central rhythm. This contrasts with the results presented in the previous chapter where strong phase-dependent influences were observed when stimulating a forewing SR pair. Direct comparison of the two studies is not possible due to a number of experimental differences. The study described in the previous chapter did not remove all phasic sensory input from the wings. The hindwing sensory nerve was left intact. Therefore, the hindwing stretch receptors as well as other proprioceptive afferents were still influencing the central nervous system. In addition, the SR afferents were stimulated at pro.n6 where they could be selectively activated. Wilson and colleagues stimulated the SRs at their projection in $n1d_2$ and almost certainly co-stimulated units from the wing chordotonal organ. Also, the experiments in the previous chapter involved stimulation of two forewing SRs whereas Wilson et al stimulated one forewing SR and the ipsilateral hindwing SR. It was important to resolve the discrepancy with these earlier studies, both because of the implication in understanding SR function and because the earlier results have often been cited as an example of purely phase-independent effects of a phasic sensory stimulus.

This chapter also addresses a second question. Namely, can the resetting of the rhythm described in the previous chapter be attributed to a direct effect of the SR afferents on the

central rhythm generator? This cannot be inferred from experiments in intact or semi-intact preparations. The resetting observed by stimulating one proprioceptor could be due to an effect on motoneurons which in turn alters movements and changes the activity in other proprioceptors. It may be the other proprioceptors which directly affect the rhythm generator. Alternately, the stimulated proprioceptor may alter the influence of other proprioceptors centrally (via presynaptic effects or effects on intercalated neurons). Such schemes could conceivably account for the inability of the previous studies to demonstrate resetting.

Before proceeding with a cellular analysis of this system it was necessary to establish that input from the SRs alone could affect the timing of the central oscillator on a cycle-by-cycle basis. If this is not so, then one cannot account for resetting in terms of only SR central connections. The study also includes consideration of the hindwing SRs, which have not hitherto been examined.

B. MATERIALS AND METHODS.

Experimental Preparations.

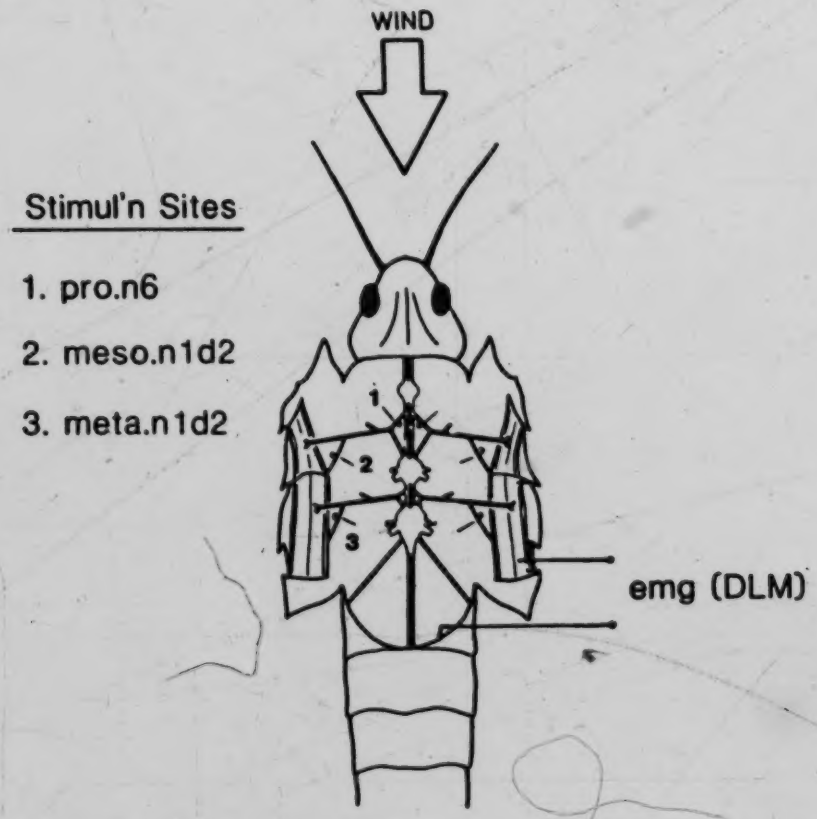
The experiments described below used one of two preparations. The first preparation ("ventral preparation") was similar to the preparation used in the previous chapter. Briefly, the animal was mounted ventral side up on a corkboard after amputating the wings and legs. It was restrained by 2 pins placed through the prothoracic cuticle and in some cases another two pins were placed through the posterior metathorax to reduce movements. The major difference from the previous preparation was that the ventral cuticle overlying the metathoracic ganglion was also removed and the first nerves of both segments were cut distally to the junction with the 6th nerve of the next anterior segment. This removed phasic sensory feedback from both the forewings and hindwings. Flight motor activity was monitored through EMG recordings from the hindwing first basalar muscle (a direct depressor of the hindwing) via a single copper wire (100μm, insulated except for the tip) placed through the ventral metathoracic cuticle. A

reference electrode was placed in the abdomen. This preparation was used to examine the effects of the forewing SRs when they were stimulated at their pro.n6 projection.

The second preparation ("dorsal preparation") was similar to that described by Robertson and Pearson (1982) for intracellular recording from central neurons during the deafferented rhythm. Following removal of the legs and wings the animals were pinned dorsal side up on a cork board. An incision was made along the dorsal midline and the thoracic ganglia exposed by removing the overlying gut and muscles. The thoracic cavity was filled with saline (NaCl-147mM, KCl-10mM, CaCl-4mM, NaOH-3mM, HEPES-10mM). This preparation is illustrated in Figure 16. In this preparation all the major branches of the wing sensory nerves (n1) are clearly visible and accessible. Sensory input from wing proprioceptors was removed by cutting the mesothoracic and metathoracic n1c's and crushing or cutting $n1d_2$ peripherally. Different pairs of SR nerves were stimulated at one of 2 sites - either at the n6 projection of the SR afferent or at its $n1d_2$ projection. Oscillator output was recorded from either the metathoracic dorsal longitudinal muscle or from the metathoracic subalar muscle. In some cases oscillator output was also monitored by intracellular recording from flight motoneurons in addition to a depressor muscle (DLM). The techniques for intracellular recording are described in the next chapter.

There was no qualitative difference between the results obtained in the two preparations. The range of frequencies observed in response to a wind stimulus was the same. The ventral preparation had the advantage that a direct comparison could be made with the experiments described in the previous chapter, as these preparations differed only in the presence of sensory input from the hindwings. In both preparations, flight activity was elicited by directing wind to the head from a tube (12mm inner diameter) placed 8cm in front of the animal. The tube was connected to a compressed air cylinder via a pneumatic relay. Wind velocities were kept in the range of 1-3m/s.

Figure 16. Schematic illustration of dorsal preparation. The dissection is described in the text. Indicated by the numbers are the various sites of stimulating electrode placement used in these experiments. Motor output was recorded from the hindwing dorsal longitudinal muscle as indicated. In other animals, depressor output was also recorded from the hindwing subalar muscle, which is not indicated in this figure. This figure also shows the flight motor nerves to be cut. This was not performed in all preparations. For further details see text.


WIND
Stimul'n Sites
1. pro.n6
2. meso.n1d2
3. meta.n1d2
1
2
3
emg (DLM)

Stimulation Procedures.

Pairs of nerves were stimulated as either a segmental pair (forewing or hindwing) or a lateral pair (one forewing SR and the ipsilateral hindwing SR). The forewing SR afferent was stimulated at one of two sites - at its pro.n6 projection or at its projection in meso.n1d_2. As previously shown, stimulation of pro.n6 selectively activates the f-$\overline{SR}$ afferent without co-stimulation of other wing afferents (see previous chapter). Selective stimulation was more difficult to achieve from meso.n1d_2. The SR is the largest axon travelling in this nerve (Gettrup 1962, Altman et al 1978) and has a lower threshold than the accompanying axons. However, the threshold differences were very small ($<$.2V). Wilson and colleagues did not establish the SR threshold and almost certainly co-stimulated the chordotonal organ afferents. Their criterion for adjusting stimulus strength was that the stimulation did not spread to the dorsal longitudinal motor nerve (meso.n1d_1). I used this same criterion in these experiments. The purpose of these experiments was to establish whether co-stimulation of other afferents was responsible for the failure of Wilson *et al* to entrain the flight oscillator with SR stimulation.

Electrodes were 75μm silver or platinum wires with an indifferent electrode placed in the haemolymph (ventral prep.) or saline (dorsal prep.) lateral to the prothoracic ganglion. Nerves were lifted on the electrodes and insulated with a petroleum jelly - mineral oil mixture. In most experiments stimuli were applied simultaneously to the pair of nerves in question via a single stimulator with the electrode pairs connected in parallel. A second stimulator was used as a trigger source. Stimuli were delivered as short trains lasting from 15-35 msec at 200-300Hz. In most cases trains were 25msec duration at 250Hz. Each stimulus pulse was 0.1msec duration. One stimulus refers to a single stimulus train. To prevent movements from dislodging the electrode, the nerve was crushed rather than cut in a number of animals. In these cases, the effectiveness of the crush was gauged by its ability to block incoming activity recorded through the stimulating electrode. In any one set of experiments both nerve crush and nerve cut were used in different animals. There was no difference in the results obtained with the two procedures. All experiments were performed at room temperature.

In these experiments, the ability of the stimulation to influence the timing of the flight rhythm was examined by testing its ability to entrain the rhythm. The ability to entrain the rhythm implies its ability to reset (Perkel et al 1964, Aschoff 1965). Stimulation was commenced coincident with the wind stimulus or at a varied time after the commencement of flight activity. The rate of stimulation was not varied during a trial except when examining the range of frequencies over which entrainment could be obtained. The oscillator output (depressor muscle spike) was then examined for any phase preference in the stimulus period. The phase-dependence of any observed effects was also tested by using time-locked stimulation. Here, stimuli were triggered by an event in the flight cycle (in these experiments, the hindwing depressor spike) and the latency between the event and the stimulus was varied. The deafferented flight rhythm is not of constant period and hence latency-constancy does not translate into phase-constancy. However such experiments can test whether or not an effect is purely phase-independent.

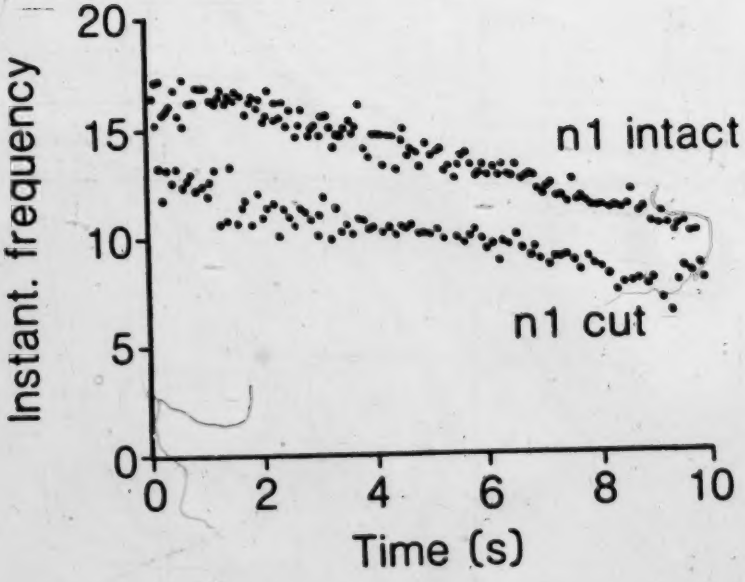
C. RESULTS.

Forewing SRs.

An important question was whether the forewing SRs could reset the timing of the central flight oscillator in the absence of all other proprioceptive input from the wings. Therefore, the experiments described in the previous chapter were repeated but with the hindwing sensory nerve (meta.n1) cut.

Figure 17 illustrates a typical effect of cutting the hindwing sensory nerves (the forewing sensory nerves had previously been cut) on the response to a windstream directed to the head in a ventral preparation. Invariably, the frequency of the rhythm decreased to values described previously for deafferented preparations (6 - 12 Hz, Wilson 1961, Kutsch 1974). There was no consistent effect on the flight sequence duration. Decreases, increases and no changes in flight duration were all observed, although decreases were the most common.

Figure 17. Effect on flight activity of cutting both hindwing sensory nerves. The metathoracic nerves1 (n1) were lesioned distal to the junction with the mesothoracic nerves6. The forewing sensory nerves (meso.n1) had previously been cut. Plot of instantaneous flight frequency versus time following the onset of a continuous wind stimulus to the head. Cutting the hindwing sensory nerves caused a further decrease in flight frequency. In this case, there was no obvious effect on the duration of flight sequences. The effects on flight duration varied from animal to animal with decreases, increases and no changes being observed.


Instant. frequency
20
15
10
5
0
0
2
4
6
8
10
Time (s)
n1 intact
n1 cut

What is the effect of time-locked stimulation of the f-SR afferents on the deafferented flight frequency? The preparation used here was similar to that described in the previous chapter except that the ventral cuticle overlying the metathoracic ganglion was also removed, which exposed the hindwing sensory nerves (meta.n1). Initially, these nerves were left intact. Two seconds after the initiation of flight by a constant windstream to the head, bilateral stimulus trains applied to the pro.n6 were triggered to occur immediately following the hindwing depressor spike on every cycle. After a number of trials (5 - 25), both meta.n1s were cut distally to their junction with meso.n6, thereby removing afferent input from the hindwing. The effects of introducing bilateral f-SR stimulation were then re-examined. An example of these results is presented in Figure 18. Before cutting the hindwing sensory nerve, the effects of f-SR stimulation were the same as described in the previous chapter. There was a rapid rise in flight frequency, beginning on the first cycle and the duration of the flight sequence was prolonged. Qualitatively similar results were obtained after removing hindwing sensory input by cutting both meta.n1s (Fig.18). However, the final frequency that was reached was reduced (11 - 14Hz vs. 16 - 20Hz). Because the initial pre-stimulus frequency was not the same in the two conditions, I examined the absolute time each cycle was reduced after introduction of the stimulation. Results from two different animals are shown in Figure 19. These represent the two extremes of result. In 4 of 6 cases the absolute time each post-stimulus cycle was shortened was less after deafferentation of both segments. Figure 19b was more typical of the rate at which the f-SRs decreased the period in deafferented preparations. In the 6 cases, the final period was 10 - 15 msec less than before stimulation, compared to 15 - 25 msec reductions in period when the hindwing afferents were left intact.

Presentation of stimulus trains at a constant rate, independently of the central oscillator, could entrain the motor output. An example of the relationship between f-SR stimulation and oscillator output during entrainment is shown in Figure 20. Elevator and depressor activity were monitored simultaneously. The former was recorded intracellularly from a mesothoracic tergosternal motoneuron (upper trace Fig. 20c,d) and the latter from the

Figure 18. Increase in flight frequency by triggered stimulation of the forewing SRs before and after cutting both hindwing sensory nerves. The forewing sensory nerves (meso.n1) had previously been cut. Two superimposed trials from the same animal showing the instantaneous flight frequency versus time. In both cases, flight activity was initiated by a constant windstream to the head. Two seconds after flight initiation, the forewing SRs were stimulated to occur every cycle immediately following the hindwing depressor spike. In the first trials, the metathoracic nerves were exposed but left intact. Commencing f-SR stimulation caused a marked elevation in flight frequency. After 10 trials, the metathoracic nerves1 were cut and the experiments repeated. The flight frequency rapidly increased but the final frequency reached was less than that seen with the metathoracic nerves left intact.

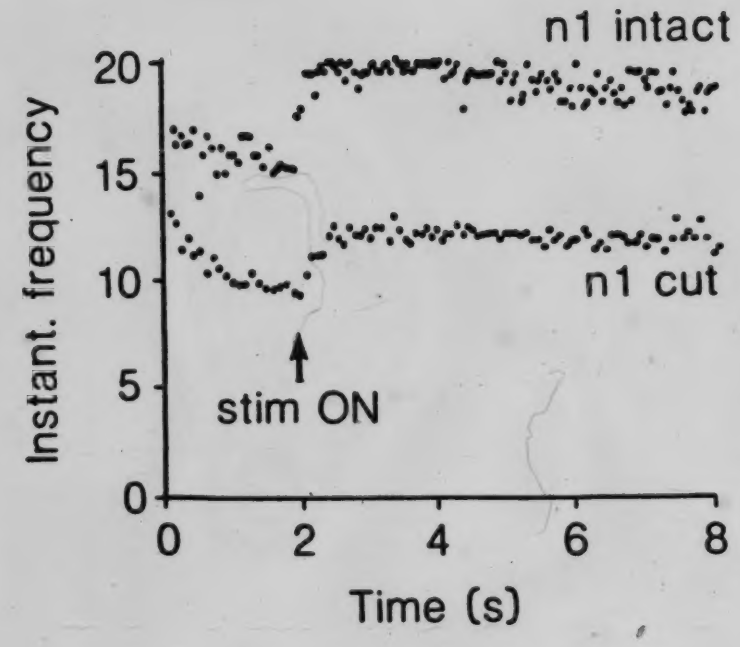

n1 intact
n1 cut
stim ON
Instant. frequency
Time (s)
0
5
10
15
20
0
2
4
6
8

Figure 19. Decrease in period caused by bilateral forewing SR stimulation before and after cutting both hindwing sensory nerves. Flight activity was initiated by a constant windstream to the head. Two seconds after flight initiation, both f-SRs were stimulated at zero msec delay following the hindwing depressor spike on every cycle. Plot of absolute time (msec) by which each successive interval is shortened relative to the interval immediately preceding the onset of f-SR stimulation. Each point is average of 10 trials before (filled circles) and after (open circles) cutting both metathoracic nerves1. Vertical bars indicate standard deviation. A and B are the same experiment in different animals, illustrating the extremes of result. In animal A, the amount by which the stimulus decreased the period was similar in both conditions. In B, cutting the hindwing nerves significantly decreased the absolute time each successive interval was shortened.

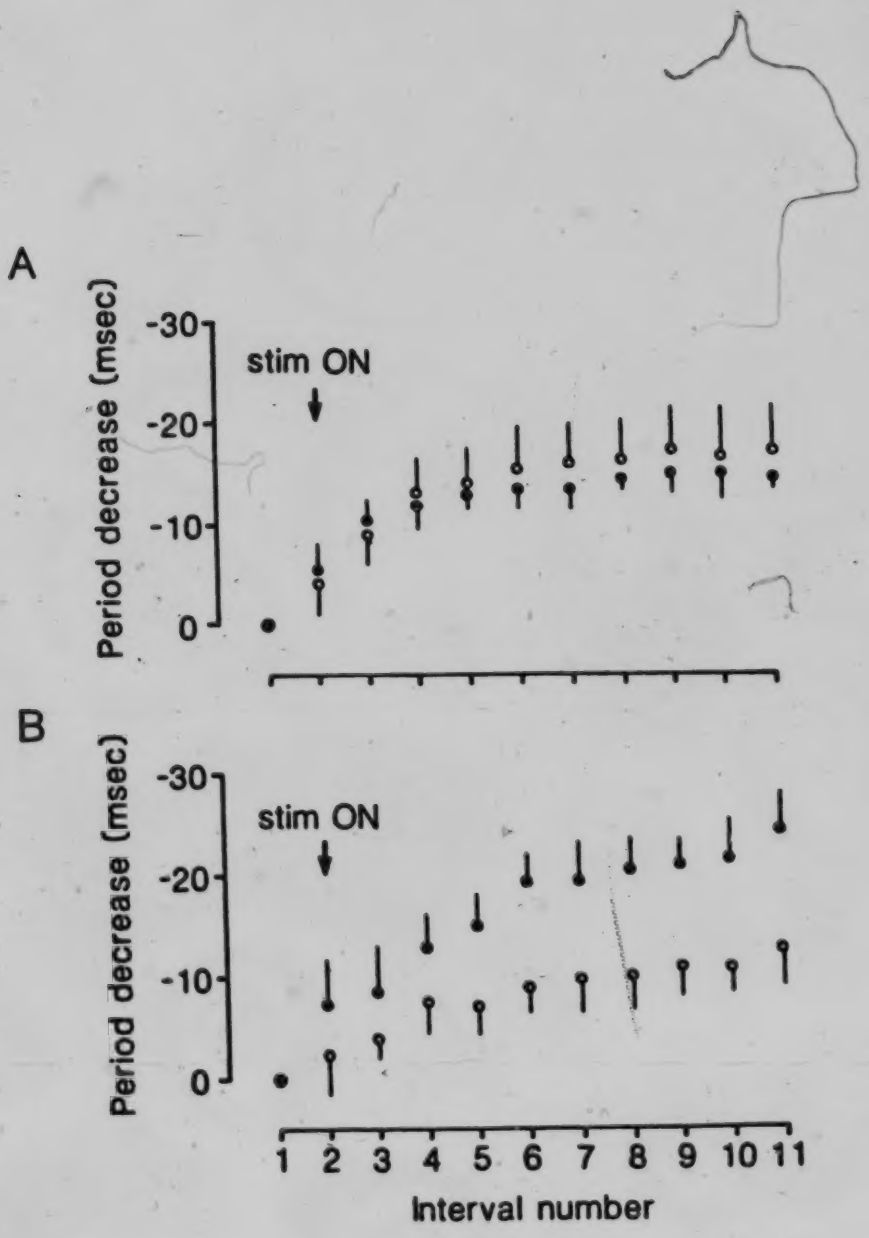
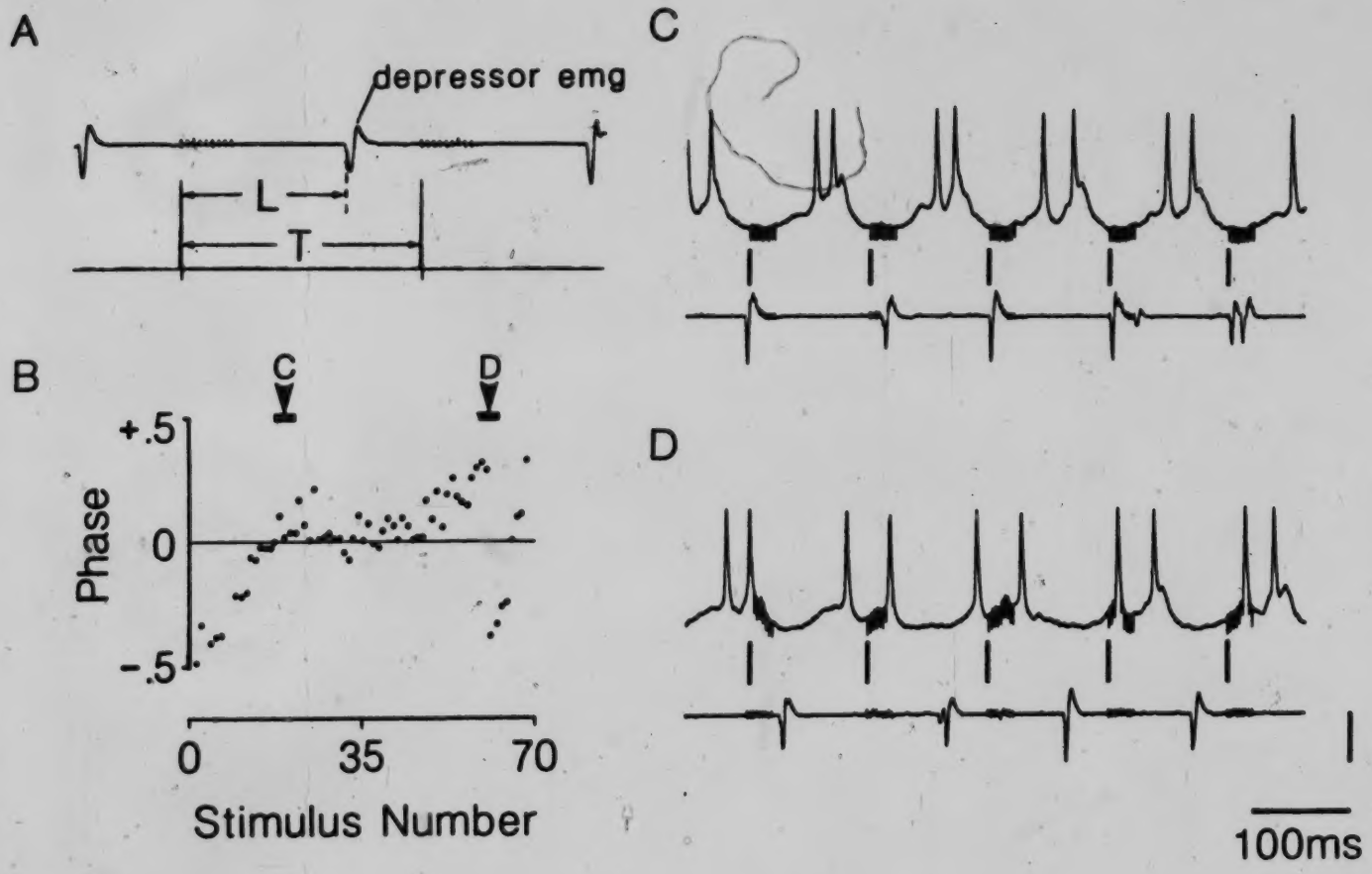

A
Period decrease (msec)
-30
-20
-10
0
stim ON
B
Period decrease (msec)
-30
-20
-10
0
stim ON
1 2 3 4 5 6 7 8 9 10 11
Interval number

Figure 20. Entrainment of the central flight rhythm by forewing SR stimulation. A. Phase was calculated as the latency (L) of the depressor spike following the last stimulus trigger pulse divided by the stimulus period (T). Zero phase was taken as the time of the stimulus trigger pulse. These values have been expressed so that depressor spikes falling in the first half of the stimulus period have phases in the range from 0.0 to +0.5, spikes falling in the latter half of the stimulus period were in the range -0.5 to 0.0. B. The phase in the stimulus cycle at which depressor spikes occurred on successive stimulus periods. The entraining frequency (8.3 Hz) was higher than the central frequency. When the depressor phase coincided with the stimulus the central frequency increased to equal the entraining frequency and phase-locking was observed for approximately 50 cycles. C. Elevator activity was recorded intracellularly from a mesothoracic tergosternal motoneuron (upper trace) in conjunction with the depressor activity seen in the dorsal longitudinal muscle (lower trace). Record taken from region indicated in (B) when the oscillator was entrained to the SR input. Stimuli fall in the hyperpolarized phase of the elevator cycle, coincident with the depressor phase. D. As in (C) but taken after entrainment has ceased at the region indicated in (B). Stimuli now move through all phases in the oscillator cycle. Calibration : Vertical 10mv.


A
depressor emg
L
T
B
C
D
+.5
0
-.5
Phase
0
35
70
Stimulus Number
C
D
100ms

metathoracic dorsal longitudinal muscle (DLM) (lower trace Fig. 20c,d). Figure 20b is a sequential phase diagram (SPD) plotting the phase of the DLM spike in the stimulus period on successive cycles. Stimulation (in this case 8.3 Hz) was commenced after the oscillator frequency was below the entraining frequency, indicated by the initial positive slope in the SPD (the depressor spike is falling at progressively later times in the stimulus cycle). When the stimulus fell close to the depressor phase of the oscillator cycle, the average oscillator frequency increased to equal the entraining frequency. During entrainment stimuli fell in the hyperpolarized phase of the elevator cycle, coincident with the depressor phase (stimulus onset indicated by the vertical bars in Fig. 20c,d). In this case, the phase relationship was maintained for approximately 50 cycles. The flight oscillator frequency then fell below the entraining frequency and stimuli occurred at progressively later phases in the stimulus period (Fig.20d), followed shortly after by cessation of the flight sequence.

It was important to establish that the phase-locking did not occur by chance, that is that the set entraining frequency did not fortuitously coincide with the intrinsic central frequency. The first argument against this was that the central rhythm in most animals did not keep a constant frequency in the absence of SR stimulation but slowed down, making the possibility of "accidental" phase-locking for more than 10 - 15 cycles improbable. To further test this, constant frequency trigger pulses were recorded without stimulating the SRs but with all other experimental conditions left unchanged. If entrainment was occurring by chance, then similar phase-locking should be observed to that when the SRs were being stimulated. However, this was not the case. No phase-locking could be detected, the rhythm continued slowing until the flight sequence ended and the depressor spikes fell across all phases in the stimulus period. Entrainment could be obtained, however, when the stimulators were connected. Entrainment was observed consistently in repeated trials and in different animals ($n>25$). Failure to entrain the rhythm was always associated with animals which flew for very short periods (10 - 20 cycles) or with failure of the electrical stimulation to elicit SR afferent spikes.

Further evidence that entrainment did not occur by chance was shown by another set of experiments. After establishing phase-locking at a given stimulus frequency the entraining frequency was increased by approximately 0.5 - 1.0 Hz to test whether the oscillator would follow the frequency increase. Figure 21 shows an example. The central oscillator was entrained to a stimulus frequency of 10.2 Hz. After about 200 cycles of phase-locking the entraining frequency was increased to 11.3 Hz. When the frequency was increased (bottom graph Fig.21), the phase-locking was momentarily lost and the depressor spike fell in all phases of the stimulus period (middle graph Fig.21). When the depressor next fell close to the stimulus, the central frequency increased and the oscillator output again became entrained to the new stimulus frequency. In some cases, the oscillator passed several times through this phase before phase-locking was re-established.

In any one animal the mean preferred phase during entrainment was the same for a given frequency in different trials. However, comparison of results from different animals showed that during phase-locking the depressor spike fell in a range of phases extending from approximately +0.1 (i.e. occurring 1/10 cycle after the stimulus) to -0.25 (i.e. occurring 1/4 cycle before the stimulus), though in most cases it fell before the stimulus. In different animals, the lowest frequency of entrainment found was 7.5 Hz and the highest 14 Hz. The majority fell within the limits of 8.5 - 12.5 Hz. In any one animal, however, the range of entrainment was low, usually 1 Hz or less (10%). This compared with a range of 4 - 6 Hz (40 - 50%) seen when the hindwing afferents were intact (previous chapter).

The central rhythm could be entrained when the f-SR afferents were selectively stimulated. Was the site of stimulation important? This was found not to be the case. The rhythm could also be entrained by stimulating the f-SR afferents at their projection in meso.nld$_2$. Results from two different animals are shown in Figure 22. In one case (Fig.22a), the intact pro.nos were stimulated with the branch into the prothoracic ganglion left intact. In the other (Fig.22b), the f-SR afferent was stimulated at its projection in meso.nld$_2$. Entrainment of the central flight rhythm is apparent in both cases (indicated by the relatively

Figure 21. The central flight rhythm can follow changes in the entrainment frequency. The central flight rhythm was entrained to bilateral f-SR stimulation at a constant rate of 10.2 Hz. The rate of stimulus trains was then increased to 11.3 Hz. The flight rhythm then increased so that its average frequency equalled the new entrainment frequency. Abscissa : stimulus cycle number. This was arbitrarily started at zero. However, phase-locking had begun over 100 cycles before this. Upper graph : instantaneous flight frequency. Indicated by the arrows are the two frequencies of f-SR stimulation. Middle graph : phase of the hindwing depressor spike (1st basalar)in the stimulus half-period. Negative phase indicates the depressor spike has occurred in the latter half of the stimulus period. Lower graph : indicates the level of the entraining frequency. This equals the rate at which stimulus trains were presented to the f-SR nerve. When the entraining frequency was increased from 10.2 to 11.3 Hz (see lower graph), phase-locking was temporarily lost. However, when the depressor spike next fell close to the stimulus the entrainment was resumed, seen in the sequential phase diagram and in the instantaneous frequency plot.

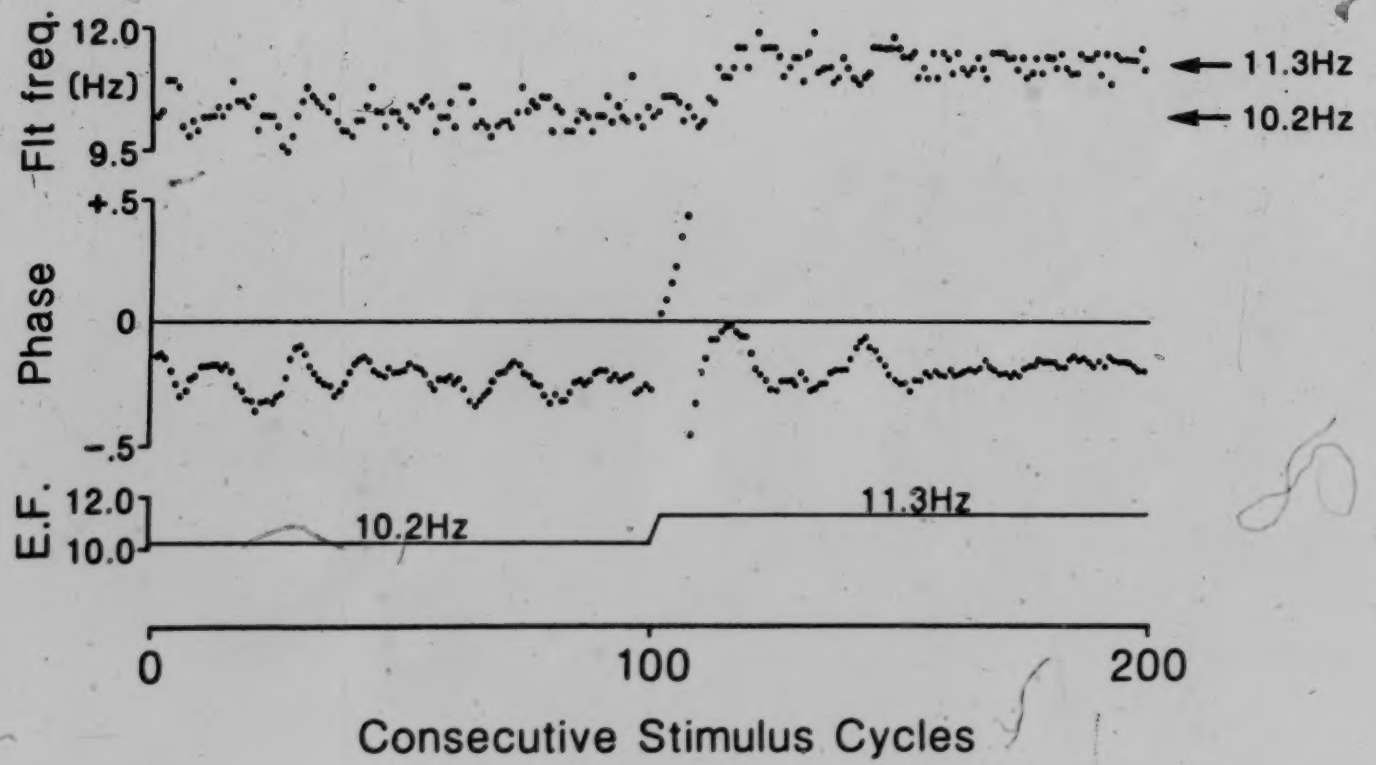
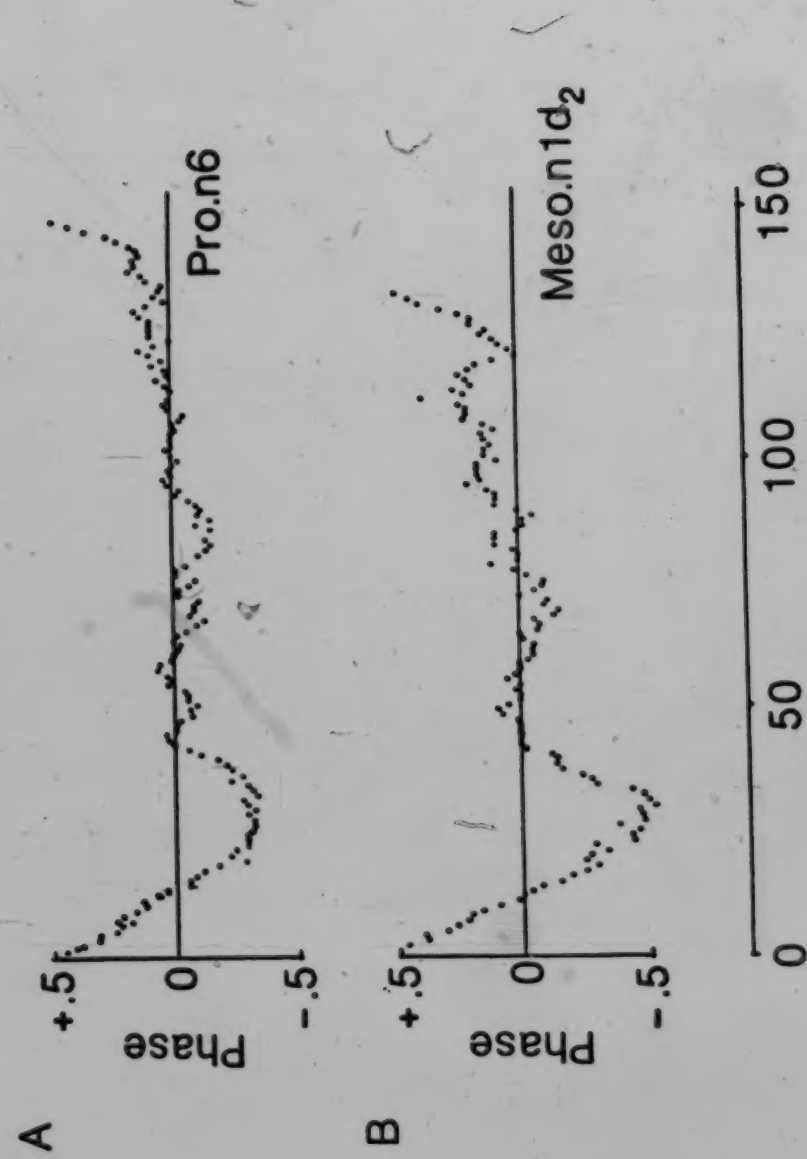

Flt freq.
12.0
(Hz)
9.5
11.3Hz
10.2Hz
Phase
+.5
0
-.5
E.F.
12.0
10.0
10.2Hz
11.3Hz
0
100
200
Consecutive Stimulus Cycles

Figure 22. The site of stimulation is not important for entrainment of the flight rhythm by forewing SR stimulation. Results from two different animals. The complete forewing SR afferent projection was stimulated at two sites. A. Stimulation of both sixth prothoracic nerves left intact. Entrainment frequency (E.F.)=9.4 Hz. B. Stimulation of both SR afferents at the peripheral projection in meso nld_2. E.F.=9.1 Hz. Both show the phase of the depressor spike in the stimulus period for consecutive stimulus cycles. The entraining frequency was set to a lower level than the initial central frequency indicated by the initial negative slope. The central oscillator frequency continues to decline slowly until it reaches the entraining frequency. There is a change from negative to positive slope before phase-locking, indicating the oscillator has first fallen below the entraining frequency.

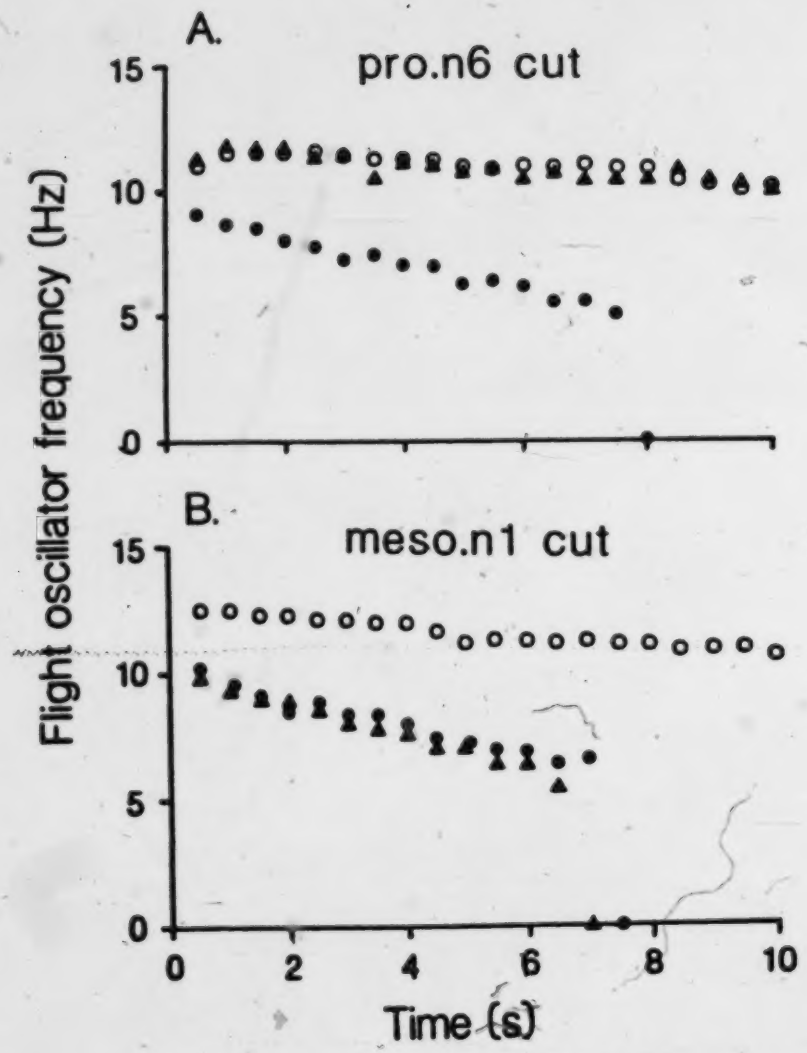

A
Phase
+.5
0
-.5
Pro.n6
B
Phase
+.5
0
-.5
Meso.n1d2
0
50
100
150
Stimulus Number

constant phase). Thus, costimulation of chordotonal organ afferents, at least in this segment and at these intensities did not prevent the occurrence of phase-locking. Also, antidromic stimulation of the dorsal longitudinal motoneurons in the prothoracic ganglion (the case in Fig.22a) did not prevent entrainment.

Of further interest from Figure 22 was the manner in which entrainment was established. In these experiments, the entraining frequency was set to a lower value than the initial central frequency. This is indicated by the negative slope in the phase diagram (the depressor spike is occurring at progressively earlier times in the stimulus cycle). The oscillator frequency continued to decline in a similar fashion to that seen when no stimulation was present. However, before phase-locking occurred there was first a change from negative slope to a positive slope in the phase diagram, i.e. the oscillator had slowed below the entraining frequency. Similar phase diagrams were observed with the selective f-SR stimulation used previously. This manner of establishing entrainment suggested that the stimulation preferably entrains the rhythm to higher frequencies.

An aid to the intracellular investigation would be to establish which of the thoracic ganglia contain the first order interneurons that mediate SR effects on the central rhythm. This was investigated by lesioning specific peripheral branches of the SR nerve and then examining the effects of SR stimulation. Electrodes were placed bilaterally on meso.nld$_2$. Stimulation was triggered to occur immediately following the hindwing depressor spike (subalar). After 5 - 10 trials, either pro.n6 or meso.n1 was cut (6 animals in each case). The stimulation experiments were repeated. The results were the same in all animals of the two groups tested. These are illustrated in Figure 23. Cutting pro.n6 had no apparent effect on the ability of the stimulation to increase the frequency and prolong the duration of rhythmic sequences, whereas all effects were abolished after meso.n1 was cut. It should be noted that stimulating meso.nld$_2$ with meso.n1 cut and pro.n6 intact selectively stimulated only the f-SR projection into the prothoracic ganglion. This was confirmed by recording pro.n6 while stimulating meso.nld$_2$. No other units except the f-SR spike were seen. The previous selective stimulation experiments

Figure 23. Effect of cutting peripheral forewing SR branches on the ability of SR stimulation to increase the central flight frequency. Two plots of frequency versus time. Each point is the average of 3 cycles every 0.5 seconds. Both f-SRs were stimulated every cycle immediately following the hindwing depressor spike (25 msec, 250/s). Initially, both peripheral branches were left intact (open circles). The response to wind alone is represented by the filled circles. A : The f-SR branch into the prothoracic ganglion was cut (triangles). There was no apparent effect on the ability of the stimulation to increase the flight frequency or to prolong the duration of flight sequences. B : The f-SR branch into the mesothoracic ganglion was cut, while the branch into the prothoracic ganglion was left intact (triangles). All effects of the stimulation were abolished, the response being very similar to that seen when only the wind stimulus was given.


A.
pro.n6 cut
B.
meso.n1 cut
Flight oscillator frequency (Hz)
15
10
5
0
0
2
4
6
8
10
Time (s)

showed that the projection into the prothoracic ganglion was not necessary to entrain the rhythm.

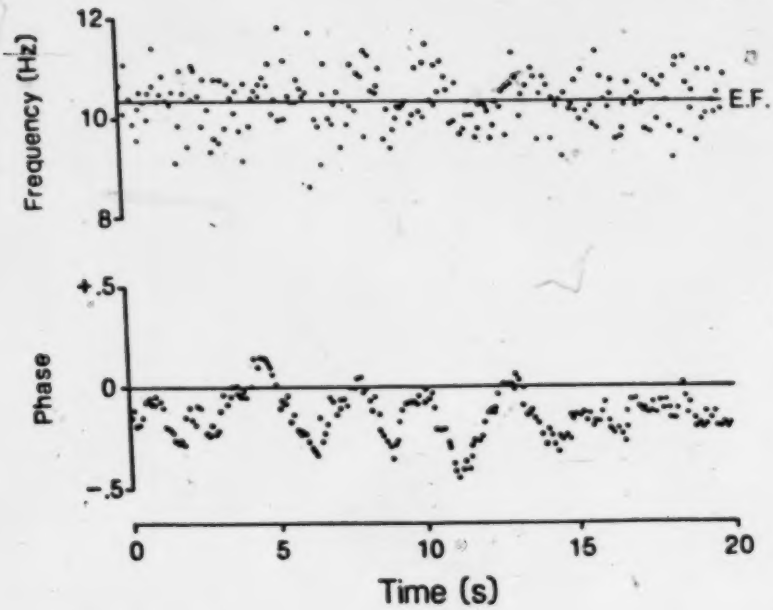
Hindwing SRs.

From the preceding, it can be seen that the forewing SRs can entrain the central flight oscillator. Is this property shared by the hindwing SRs (h-SRs)?

The peripheral nerve arrangement of the hindwing SR afferent made it more difficult to study than the forewing SRs. The length of meso.n6 was usually too short to enable good electrode placement and also raised questions of the stimulus also exciting units in the nearby meta.n1. Therefore, most experiments were performed stimulating the h-SR axon at meta.n1d$_2$ (n=5). However, in the few cases where meso.n6 was stimulated (3 animals) the results were in agreement with those presented here. Figure 24 shows an example of the sequential phase diagram resulting from bilateral stimulation of meta.n1d$_2$. The results were similar to those found for f-SR entrainment. Constant frequency stimulation could couple the central rhythm and the phase-preference observed was over the same range of phases as found for f-SR stimulation. However, one difference observed with h-SR stimulation was that entrainment tended to be less stable. A larger range of phases were covered during the coupling period, although there was no cycle skipping. Sometimes, oscillations in the flight rhythm frequency would develop (seen in Fig.24). These were also occasionally seen with forewing SR stimulation. The rhythm could follow changes in the entrainment frequency, as was found for forewing SR stimulation (not shown).

Finally, in considering the influences of the hindwing SRs on the flight rhythm, the effects of lesioning one or other of the peripheral h-SR nerves were examined. This was similar to the experiments described above for the forewing SR except that in this case either meso.n6 (2 animals) or meta.n1 (5 animals) were cut bilaterally. The hindwing SRs were stimulated bilaterally at meso.n1d$_2$ triggered every cycle from the hindwing depressor spike (subalar). As can be seen from Figure 25, cutting meso.n6 had no effect on the ability of the h-SRs to

Figure 24. Entrainment of the central flight rhythm with bilateral stimulation of the hindwing SRs. Both hindwing SRs were stimulated at the projection in meta.n1d;. Stimulus trains were presented at a constant rate of 10.3 Hz during the deafferented rhythm initiated by a constant windstream to the head. Upper graph : Instantaneous frequency of the deafferented rhythm as a function of time. The entraining frequency (E.F.) is indicated by the solid line. Bottom graph : The phase of the hindwing depressor spike (subalar) in the stimulus period as a function of time. Note the occurrence of slow oscillations in the flight frequency. This often alternated with periods of fairly steady phase-locking.


12
10
8
Frequency (Hz)
E.F.
+.5
0
-.5
Phase
0
5
10
15
20
Time (s)

increase the flight frequency. On the other hand, cutting meta.n1 but leaving the projection to the mesothoracic ganglion intact abolished all effects of the stimulation. As might be expected from this result, cutting meso.n6 did not prevent the stimulation from being able to entrain the rhythm. The rhythm could not be entrained when only the mesothoracic projection was left intact. This result suggests that the increase in frequency evoked by hindwing SR stimulation is mediated by first order interneurons in the metathoracic ganglion.

Lateral SR Pair.

The preceding results showed that stimulation of each segmental pair of SRs is capable of entraining the central flight rhythm in the absence of other proprioceptive feedback from the wings. Neither deafferentation nor the site of stimulation prevented the stimulation from entraining the central rhythm. Wilson and colleagues found that stimulating a lateral pair could not usually entrain the rhythm but, when it did, there was a disruption of the rhythmic motor output. In this section, the experiments of Wilson and colleagues were repeated by stimulating one forewing SR and the ipsilateral hindwing SR. Most were performed by stimulating at meso.n1d_2 and meta.n1d_2, firstly because this site was used by Wilson *et al* and secondly because of the difficulties in stimulating meso.n6.

The first experiments were a direct test of the hypothesis that the SRs exert a purely phase-independent excitatory influence on the central oscillator. The lateral pair of SRs were stimulated with the stimulus triggered to occur at a set latency following the hindwing depressor spike (hindwing subalar). If the effects are phase-independent then the latency of the stimulation with respect to the depressor should have little effect on the overall flight frequency. Figure 26 shows an example of stimulation triggered immediately following the depressor spike (0 msec delay) compared to the frequency profile seen when stimuli were timed to occur 45 msec after the depressor spike (approximately midway through the cycle). When stimuli coincided with the depressor burst, there was an increased frequency and a prolongation of the flight sequence. On the other hand, when stimuli occurred at later times in the cycle

Figure 25. Effect of cutting peripheral hindwing SR branches on the ability of hindwing SR stimulation to increase the central rhythm frequency. Two plots of frequency versus time. Each point is average of 3 cycles every 0.5 seconds. Both h-SRs were stimulated every cycle immediately following the hindwing depressor spike. Initially both peripheral branches of the h-SRs were left intact (open circles). The response to wind alone is represented by the filled circles. A : The h-SR branch into the mesothoracic ganglion was cut (triangles). There was no apparent effect on the ability of the stimulation to increase the flight frequency. B : The h-SR branch into the metathoracic ganglion was cut, while the branch into the mesthoracic ganglion was left intact (triangles). All effects of the stimulation were abolished, the response being very similar to that seen when only the wind stimulus was given.

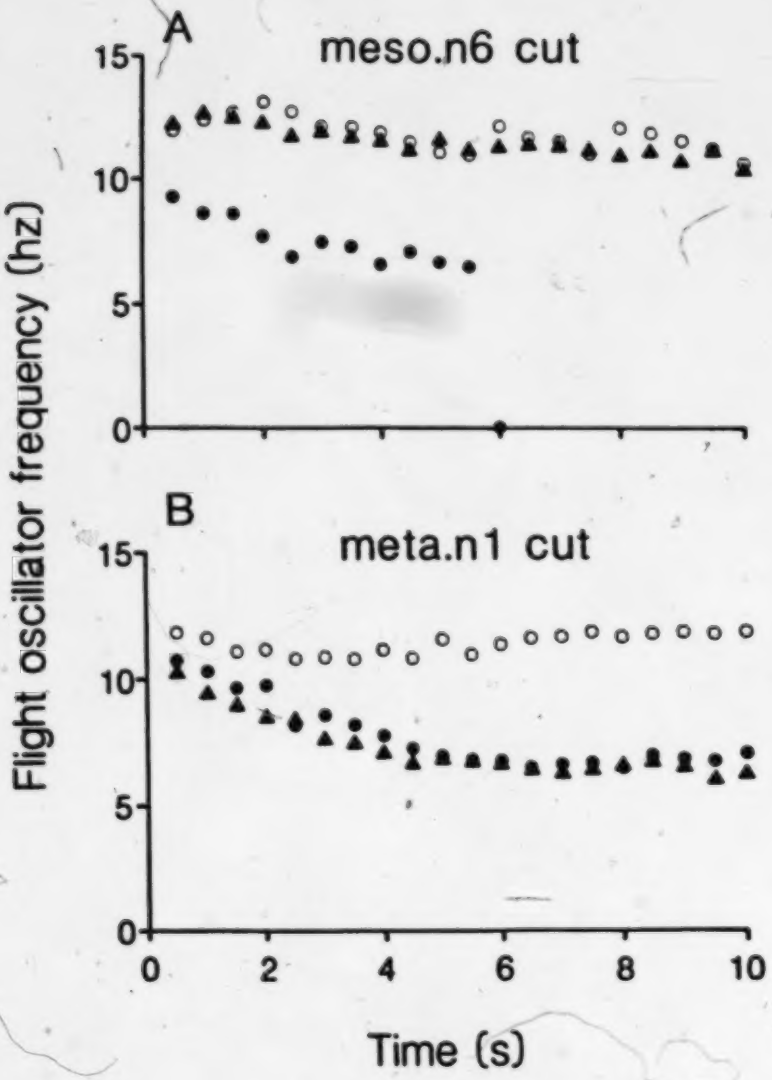
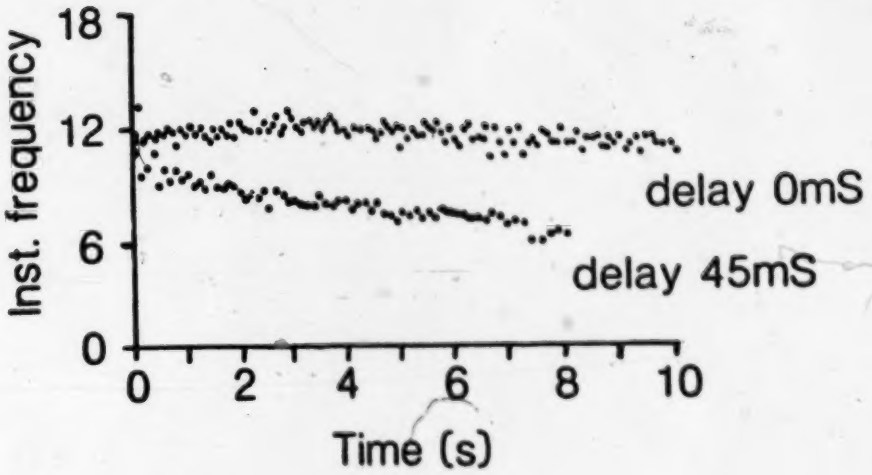

A
meso.n6 cut
B
meta.n1 cut
Flight oscillator frequency (hz)
15
10
5
0
0
2
4
6
8
10
Time (s)

Figure 26. Phase-dependent effects of lateral SR pair stimulation on the central flight rhythm. Data from two separate trials in the same animal. SR stimulation was triggered from the depressor burst on every cycle. In one case stimuli occurred immediately following the depressor burst (0 msec delay), while in the other stimuli were timed to occur 45 msec after the depressors (45 msec delay). The frequency and duration of the flight sequence were enhanced in the former condition compared to that seen at 45 msec delay. The frequency profile seen in the latter case was very similar to that seen when no SR stimulation was presented. Stimulus site : meso.n1d$_2$ and the ipsilateral meta.n1d$_2$.

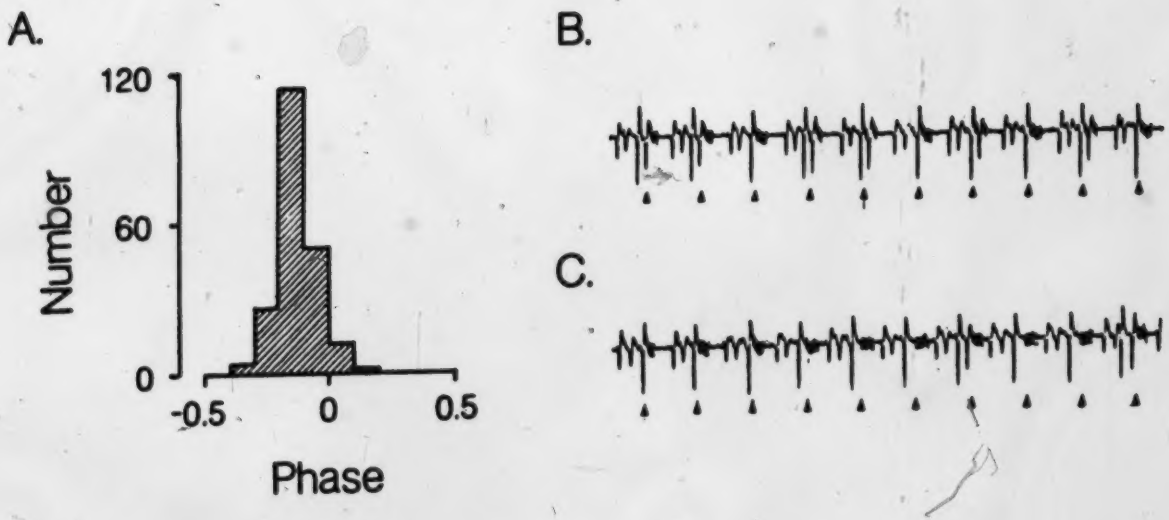

Inst. frequency
18
12
6
0
0
2
4
6
8
10
Time (s)
delay 0mS
delay 45mS

there was no obvious effect on the flight frequency, the profile observed being very similar to that seen when no SR stimulation accompanied the wind stimulus (not shown). These results were observed in all 12 animals tested and also using the same stimulus train parameters as employed by Wilson and co-workers (5 pulses at 200/s, measured from Wilson and Gettrup 1963). Thus, stimulation of a lateral pair of SRs does exhibit phase-dependent effects. The maximum frequency reached with a lateral SR pair was of similar magnitude to that observed with triggered stimulation of either segmental pair of SRs. I did not find that imposing a specific phase relationship between SR stimulus and the oscillator disrupted the rhythmic motor output.

However, despite these phase-dependent effects, presentation of stimulus trains at a constant rate independently of the central oscillator in nearly all cases could not entrain the central rhythm in a 1:1 fashion. In only one preparation was consistent phase-locking observed in consecutive trials. The results from this animal are shown in Figure 27. Although seen in only one preparation these results were of interest. Firstly, the phase preference was in the same range as observed for segmental stimulation as can be seen in the frequency histogram (Fig.27a). In three consecutive trials the mode of this distribution was the same. Secondly, there was no disruption of the rhythmic motor output during entrainment. Wilson and Wyman (1965) reported occasionally seeing a strong phase preference but that when this occurred, the rhythm would become disrupted. Figure 27 shows the EMG records obtained in the same trial. The large negative going spikes are from the ipsilateral (to the stimulating electrodes) mesothoracic subalar muscle, the smaller preceding potentials being pick-up of nearby elevator activity. Figure 27c was taken some 60 cycles later than the record in Figure 27b. There was no disruption of the rhythm, nor was there any obvious general increase in elevator and depressor activity as reported by Wilson *et al*.

Except for this one case, lateral SR pair stimulation could not entrain the central rhythm (n=15). Generally, as the oscillator frequency slowed to approach the entraining frequency some "attraction" could be seen when the stimuli fell close to the depressor phase but

Figure 27. Entrainment of the central flight rhythm by stimulation of a lateral pair of SRs. A: Frequency histogram of phase in the stimulus period at which the depressor spikes fell during entrainment in one trial. B and C: Examples from the same trial of the motor output (depressor EMG) recorded during entrainment. The record in C was taken 60 cycles later than the record in B. The large negative going spikes were recorded from the ipsilateral hindwing subalar muscle. Note that there was no tendency for the discahrge to become arrythmic. The time of onset of each stimulus train is indicated by an arrowhead. The small transients seen in the EMG records are stimulus artifacts. Entrainment frequency = 9.0 Hz.


A.
120
60
0
Number
-0.5
0
0.5
Phase
B.
C.

this was not capable of coupling the oscillator for more than 5 - 20 cycles, with the oscillator continuing to slow below the entraining frequency. Even in cases where the oscillator tended to maintain a relatively constant frequency, lateral SR stimulation did not give absolute coordination, although in some (but not all) cases, indications of relative coordination could be seen (Fig.28). Hence an ipsilateral pair of SRs were much weaker at coupling the central flight oscillator than a segmental pair.

Thus far, the experiments have involved placing the entraining frequency close to the oscillator frequency. Wilson *et al* reported that constant frequency stimulation of the SR nerves produced a slow increase in the flight frequency with no phase-preference shown between the stimulus and the oscillator output. However, Wilson and Gettrup (1963) specifically state that these effects were observed when the entraining frequency was considerably higher than the central frequency. Measurements from that paper (their Figure 11) show an entraining frequency of 16 Hz while the oscillator frequency was 12 Hz. It has already been shown that if the SR input occurred at the oscillator frequency (the natural situation for a proprioceptor) then the effects on the central rhythm were phase-dependent. I examined the effects on the central rhythm of high frequency SR stimulation. As described previously, the usual response of the deafferented rhythm to a constant windstream applied to the head was an initial frequency of 10 -14 Hz which gradually declined over time to 6 -8 Hz followed by cessation of the rhythm. If onto this type of activity were imposed high frequencies of lateral SR trains (14 -17 Hz), the usual observation was that the sequence was somewhat prolonged (ca.25%) but not to the degree observed if that stimulation were triggered to occur immediately following the depressor spike. The rate at which the central frequency declined was reduced rather than there being an actual increase in frequency. However, in a few cases where the deafferented rhythm was maintained for long times (3 cases), it was possible to repeat the results of Wilson et al, the difference being that the time to reach maximum frequency was slower (>100 cycles to maximum vs. 35 -40 cycles) (Figure 29). However, it was only under conditions of a high rate of stimulus presentation that these effects could be observed. As was also found by Wilson et

Figure 28. Evidence for relative coordination of the flight rhythm with stimulation of a lateral pair of SRs. Upper graph : Instantaneous frequency versus time. The rate of SR stimulation (entraining frequency,E.F.) is indicated by the solid line. Bottom graph : Sequential plot of the phase that the depressor spike occupies in the stimulus period. Note the occurrence of inflections in the phase diagram (arrows) when the depressor spike fell close to the time of the stimulus.

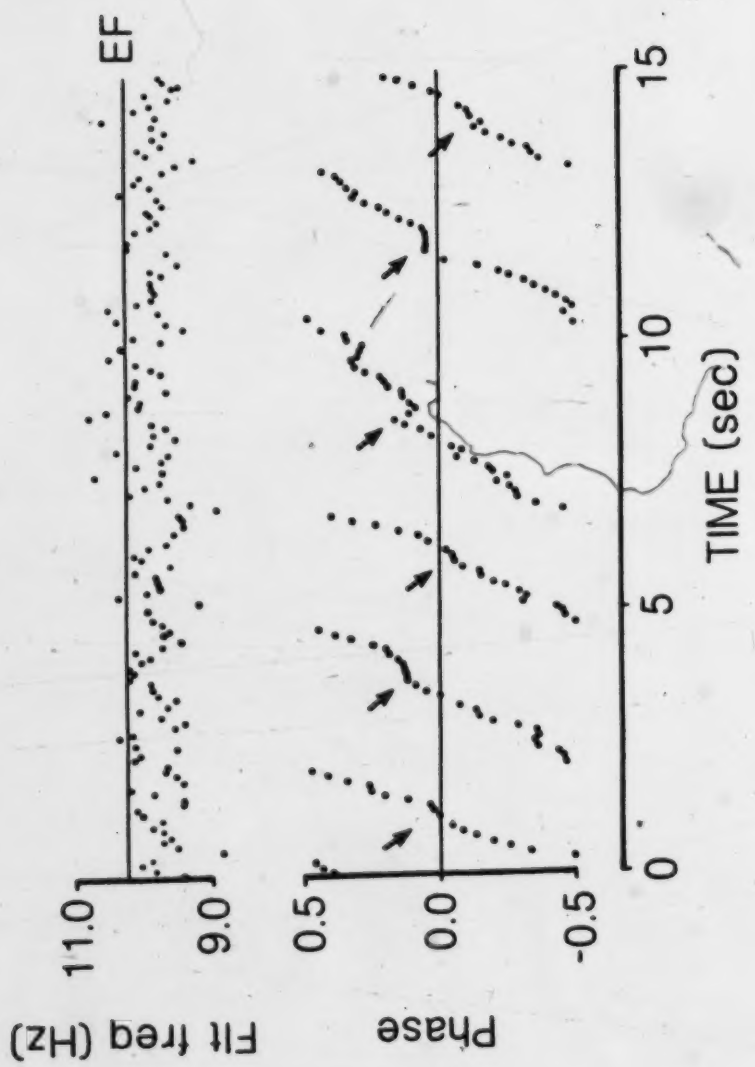
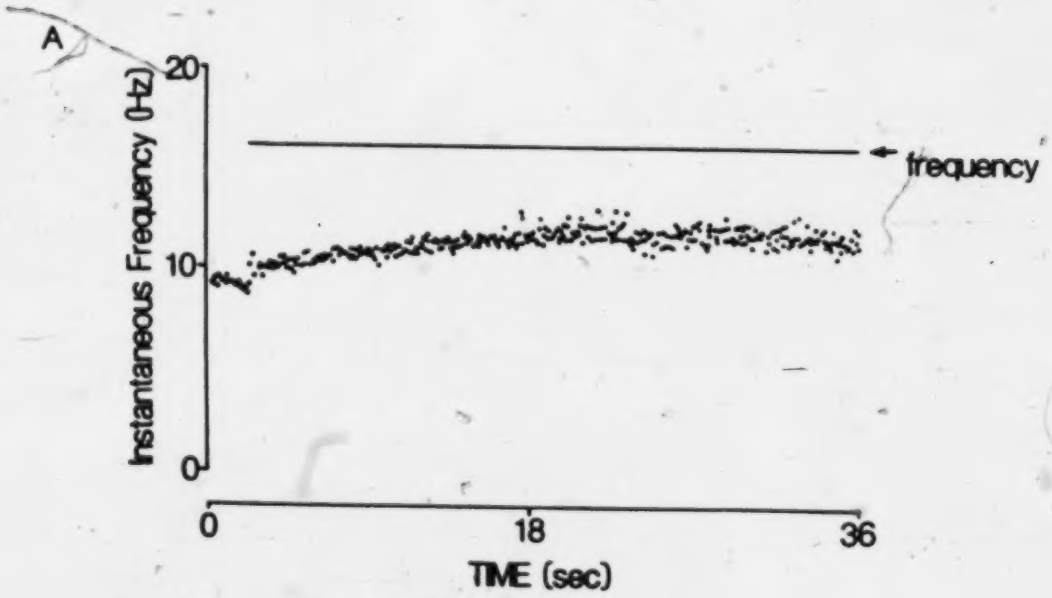
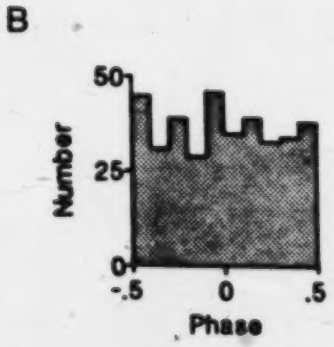

EF
11.0
9.0
Flt freq (Hz)
0.5
0.0
-0.5
Phase
0
5
10
15
TIME (sec)

Figure 29. Slow increase in central flight frequency by stimulation of a lateral pair of SRs. A: Instantaneous frequency of the flight rhythm versus time. A lateral pair of SRs (one forewing SR and the ipsilateral hindwing SR) were stimulated with stimulus trains at a rate of ca.16/s (indicated by the solid horizontal line). Site of stimulation was $n1d_2$. There was a small quick increase in frequency at the onset of stimulation followed by a more gradual increase over the following 15 seconds. B: Histogram of the phase in the stimulus period at which the the depressor spikes occurred for the duration of the stimulation shown in A. Note the fairly even phase distribution. This result is very similar to that described by Wilson and Wyman (1965).


A
Instantaneous Frequency (Hz)
20
10
0
frequency
0
18
36
TIME (sec)


B
50
25
0
Number
-.5
0
.5
Phase

al, there was no apparent phase-preference (Fig.29b).

D. DISCUSSION.

Qualitatively, the effects of forewing stretch receptor stimulation on the deafferented rhythm were similar to those described in the previous chapter for the same stimulation but with the hindwing sensory nerve left intact. Stimulation of both f-SR afferents could entrain the central rhythm. The preferred phase during entrainment was such that stimuli fell close to the depressor phase of the flight cycle although they had a tendency to occur somewhat later. However, the potency of the f-SRs as a resetting stimulus appeared weaker in the absence of sensory input from the hindwing. This was seen as a reduction in the range of entraining frequencies from 4 - 6 Hz to 1 Hz and a decreased shortening of the flight period with time-locked stimulation. Thus, the presence of intact sensory input from the hindwing facilitates the ablility of the f-SRs to alter the timing of the flight cycle. The identity of these receptors is unknown. Whether other forewing afferents can facilitate f-SR effects is also unknown. One possibility is that the increased effectiveness is due to summation of the effects of the f-SRs with those produced by the h-SRs. There are two arguments against this. In several animals, I stimulated all four SRs. This did not result in higher frequencies than was seen with stimulation of only two SRs. Secondly, lesion studies have shown that ablation of two SRs caused only a slight reduction in flight frequency whereas ablation of all four SRs reduces it to 50% of its intact value, close to the frequency seen in deafferented animals (Wilson and Gettrup 1963). This suggests two SRs are only slightly less effective than four SRs in increasing the flight frequency. The lesion experiments suggest that two SRs are necessary to obtain the flight frequency seen in intact animals. These experiments showed that they are not sufficient to do so. Triggered stimulation usually only increased the frequency to 12Hz and never above 14Hz.

This study goes part of the way towards explaining the discrepancy between the results presented in the first chapter and those of Wilson and colleagues. The failure of these workers

to see absolute entrainment of the flight rhythm lay in the fact that they stimulated an ipsilateral pair of SR afferents. This pair could only rarely entrain the flight rhythm in these experiments. Relative co-ordination was sometimes, but not always, apparent. Wilson *et al* judged phase preference from histograms of phases that occur during the stimulation. Such weak relative co-ordination may have been missed using this method. However, it cannot be ruled out that the species difference may also be a factor. In the present experiments a single forewing SR could only rarely entrain the central oscillator. Thus, to consistently obtain entrainment, SR input needs to come from both sides of the animal. A similar situation has recently been reported in locust flight for resetting of the rhythm by stimulation of the wing campaniform sensilla (Horsmann and Wendler 1985).

In considering Wilson *et al*'s finding of a tonic excitatory input, it is important to look at the conditions under which these effects were produced. These workers found that the SRs had to be stimulated at a considerably higher rate than the central oscillator to produce this slow increase in frequency. This is an unphysiological situation. Being proprioceptors activated by the oscillator, SR bursts cannot occur at a higher frequency than that of the oscillator. If the SRs were stimulated at the same frequency (by triggering the stimulus from the oscillator output) then the effects exhibited a phase-dependence, although this pair of SRs was only weakly able to attract a free-running flight rhythm. However, such high frequency stimulation could produce a slow rise in flight frequency with no apparent phase-dependence. Also, it was found that the increase in frequency produced under these circumstances was considerably slower than reported by Wilson et al. This may have been due to a species difference or differences in the level of arousal of the preparations. However, this slow effect definitely exists. The question arises whether this effect reflects a property of the oscillator itself or a general excitation of the central nervous system.

The following chapters will be concerned with the influence of the SRs on central flight interneurones. The results from the present and the previous chapters suggest several questions around which to view these projections. They show that the SRs can reset the central rhythm,

albeit weakly. Thus an analysis of SR projections onto interneurones can lead to an understanding of the mechanisms of resetting. Why is a bilateral input a stronger entraining stimulus than an ipsilateral SR pair? Both fore- and hind-wing SR pairs can entrain the rhythm. Do they do so via a common set of interneurones? What are the mechanisms by which high frequency SR stimulation causes a slow increase in the flight frequency?

IV. SR CONNECTIONS TO FLIGHT NEURONS.

A. INTRODUCTION

The experiments described in this chapter examined the influence of the SRs on single identified neurons in the locust thoracic ganglia. The chapter begins with a study of the changes in the activity seen in depressor and elevator flight motoneurons induced by SR stimulation. Burrows (1975) described connections between the stretch receptors and flight motoneurons in quiescent preparations of the closely related locust species, *Schistocerca gregaria*. He found that both forewing and hindwing SRs evoked constant short-latency EPSPs in ipsilateral depressor motoneurons and slightly longer latency IPSPs in elevator motoneurons. Möhl (1985c) stimulated the SR afferent in intact tethered flying *Locusta*. He found that SR stimulation could advance the time at which two forewing depressor muscles (the first basalar and subalar) fired and also delay the time at which the following elevator EMG occurred. He concluded that the connections seen in *Schistocerca* also were true for *Locusta*. I have examined the connections from the SRs to flight motoneurons in *Locusta* directly by intracellular recording. This revealed that the SRs do evoke short-latency EPSPs in depressor motoneurons although their distribution to particular motoneurons was slightly different from those observed in *Schistocerca* by Burrows (1975). A more important difference, however, was that the SRs did not evoke IPSPs in elevator motoneurons. The study then proceeded to examine the changes in flight motoneuron activity caused by SR stimulation during the central flight rhythm. The SRs altered the activity of both elevator and depressor motoneurons. In depressors, the level of repolarization after each rhythmic depolarization was reduced. In elevators, SR stimulation decreased the number of spikes elicited in each cycle.

The first stage of understanding the mechanisms by which the SRs influence the central flight pattern is to identify those interneurons which receive connections from the SRs. This is described in the second part of this chapter. The SRs were found to evoke short-latency EPSPs in a large number of thoracic interneurons. One interneuron was found which responded with

an IPSP to each SR afferent spike. Where tested, individual interneurons received convergent input from the SRs of more than one wing, suggesting that SR mediated adjustments of motor output are on the basis of information received from several wings rather than each wing being controlled separately. The majority of connections were to interneurons depolarized in phase with depressor muscle spikes. In one case the ability of the SRs to change the rhythmic activity of the interneuron was reduced because the neuron was already strongly depolarized when the SR input occurred. However, other depressor interneurons were more strongly influenced by the SR stimulation. This could prolong the duration of their bursts and increase their firing frequency. However, the SRs not only connected to depressor interneurons but also formed strong connections to neurons active in elevator phase or, in one case, to a neuron not driven at all by the central oscillator. In several cases it was shown that the rhythmic activity of interneurons was radically altered in the presence of SR input.

B. MATERIALS AND METHODS.

The preparation used for intracellular recording during expression of the central flight rhythm was similar to that developed by Robertson and Pearson (1982). The dissection was described in the previous chapter (the "dorsal preparation"). The flight motor output was recorded from either the dorsal longitudinal muscles (indirect depressors) or from the hindwing subalar muscle (direct depressor). In the latter case, the metathoracic tergum and wing stump were removed. This exposed the subalar muscle allowing easier implantation of the EMG electrode and also reduced movements of the ganglia via the attached motor nerve (meta.n4). All nerves to other flight muscles were cut as were the n1c branches of the sensory nerve, which carry afferents from the tegula and campaniform sensilla.

The meso- and meta-thoracic ganglia were raised on a rigid stainless steel plate. Intracellular recording electrodes were filled with Lucifer yellow and would have had a resistance of between 40 and 60 megohms if filled with potassium acetate. Interneurons were characterized physiologically by their activity during the deafferented flight rhythm and by their

response to SR input. Their anatomical characteristics were established by injection of Lucifer yellow for 3 - 5 min with a constant current of -3 to -5 nA. Ganglia were fixed in 4% paraformaldehyde for 30 min, dehydrated and cleared in methyl salicylate. Cells were viewed as whole-mount, under epifluorescence. Some recordings were made with electrodes filled with potassium acetate (1M). These electrodes were used to study neurons which could unambiguously be identified by their physiology and location in the ganglion.

Initially, in identifying neurons receiving short-latency connections from the wing SRs, the SR nerves were left intact. Monopolar extracellular hook electrodes (75μm Ag or AgCl) were placed either on pro.n6 or meso.n1d$_2$ to record the forewing SR (f-SR) afferent spike and/or on meta.n1d or meta.n1d$_2$ to record the hindwing SR afferent spike (h-SR). Insulation was provided by coating the nerves on the electrode with petroleum jelly. In most cases, segmental pairs of SRs (either f-SR or h-SR) were recorded in any one preparation. In others, one f-SR and the ipsilateral h-SR were both monitored. Usually, the SRs spontaneously discharge at a low rate (0.1 - 1.0 Hz) and connections were identified by correlating PSP's with the extracellularly recorded SR afferent spike. Stretch receptor spikes could also be elicited by raising the wing stump. All connections have been confirmed in this manner. The excitatory postsynaptic potentials (EPSPs) described here all occurred at a constant short latency (4msec or less) following the recorded SR spike. The forewing SR connections could also be identified by stimulating pro.n6. In these cases, the SR nerves were cut peripherally. Intracellular recordings from the SR afferents showed that the conduction time into the thoracic ganglia accounted for all but 0.5 to 1.5 msec of the EPSP latency. Where tested, these EPSPs also followed one for one with SR spikes occurring at 100/s (by electrical stimulation or rapid movements of the wing stump).

Of particular interest in these experiments was to examine the responses of neurons to trains of stimuli delivered to the forewing SR afferent. In most cases, the site of stimulation was the sixth prothoracic nerve. In these experiments, stimulus train parameters were as described in the preceding chapters. One problem with this site of stimulation was that, with

repetitive stimulation, the SRs would fail to follow the imposed stimulus train frequency. This was partly due to the site of stimulation, possibly because of conduction block at the pro.n6 - meso.n1 branch point. Therefore in later experiments the SRs were stimulated at meso.n1d$_2$. Here, the overlying dorsal longitudinal muscle was removed to expose sufficient length of the nerve. A second set of hook electrodes were placed on each meta.n1d to record the incoming activity elicited by the stimulation. The strength of the stimulus was set to the threshold for eliciting 6 SR afferent spikes at 300 Hz. The threshold was monitored and adjusted throughout the experiment. It was not always possible to stimulate the SRs without also stimulating other afferents. Usually in these cases other units were seen to fire once at the beginning of the stimulus train. If non-SR units occurred throughout the train the preparation was discarded. Further separation of the SRs from other units could sometimes be obtained by increasing the ambient temperature with a heat lamp to 26°C - 28°C. All results presented in this chapter using this site of stimulation have either been observed with pro.n6 stimulation or when the SRs were selectively stimulated from meso.n1d$_2$.

C. RESULTS.

Connections of SRs to flight motoneurons.

Depressor Motoneurons

The distribution of SR connections to flight motoneurons are summarized in Table I. Here they are compared to the results seen in *Schistocerca* (Burrows 1975, Peters et al 1985). Results were obtained from 50 intracellular recordings of depressor motoneurons. The neurons listed in Table I refer only to motoneurons ipsilateral to the SRs. No connections were found with contralateral motoneurons. The general pattern of connection was that the hindwing SRs connected to both ipsilateral forewing and hindwing depressor motoneurons whereas the forewing SRs connected only to forewing depressor motoneurons. The one exception to this rule was that the hindwing SRs did not connect to

TABLE 1 - Comparison of distribution of EPSPs to depressor motoneurons in *Locusta* and *Schistocerca*. *Schistocerca* entries derived from data of Burrows (1975) and Peters et al (1985).

Constant short-latency EPSPs indicated by (+).

No connection indicated by (-).

No entry indicates connection not tested.

TABLE I

MOTONEURON		Forewing SR		Hindwing SR	
		Locusta	Schist.	Locusta	Schist.
FOREWING					
PRO.					
	81	+	+	-	-
MESO.					
	81	+		+	
	97	+	+	+	+
	98	+	+	+	+
	99	+	+	+	+
HINDWING					
MESO.					
	112	-	+	+	+
META.					
	112	-		+	-
	127	-	+	+	+
	128	-	-	+	+
	129	-	-	+	+

the forewing dorsal longitudinal motoneurons in the prothoracic ganglion (the h-SR does not project to this ganglion). This pattern was slightly different from that described in *Schistocerca* by Burrows (1975). In that species, the f-SR evoked EPSPs in two hindwing depressor motoneurons - the hindwing dorsal longitudinal motoneurons located in the mesothoracic ganglion and the hindwing first basalar motoneuron. Otherwise, the pattern of connections between the SRs and the ipsilateral depressor motoneurons in *Locusta* was the same as described by Burrows (1975) in *Schistocerca*. However, there was another apparent discrepancy between the two species in respect of depressor connections. In *Locusta* the hindwing SR evoked EPSPs in the single hindwing DL motoneuron in the metathoracic ganglion (n=3). This connection was not tested by Burrows (1975). Peters et al (1985) found no sign of synaptic connection between the h-SR and this motoneuron at the ultrastructural level in *Schistocerca*. There is the possibility that these EPSPs were not monosynaptic. However, they occurred at a constant, short latency following the h-SR afferent spike. They followed frequencies of 50 Hz (higher SR spike frequencies could not be elicited) and the latency was the same as that recorded in the hindwing first basalar motoneuron which was proved monosynaptic in *Schistocerca* by Peters et al (1985).

With only one exception the EPSPs evoked in depressor motoneurons were small, usually 1mV or less. This contrasted with the much larger amplitude EPSPs evoked in interneurons (see below). The one exception was the forewing SR to the forewing first basalar connection which was usually of the order of 2mV. The hindwing SR connection to the hindwing first basalar was only 1mV which was smaller than the values of 3 - 4 mV obtained by Burrows (1975) in *Schistocerca*.

Stimulating the SRs during flight activity had a conspicuous effect on the depressor motoneurons. This was shown by stimulating both forewing SRs so that they occurred immediately following the hindwing subalar spike. Normally during the deafferented rhythm the membrane potential oscillations in depressor motoneurons are superimposed on a small tonic level of depolarization (Hedwig and Pearson 1984). This level of

depolarization increased markedly upon presentation of SR stimulation. Examples from two forewing depressor motoneurons (the forewing dorsal longitudinal and the first basalar) are shown in Figure 30. The increase in the baseline depolarization began immediately upon commencement of SR stimulation. Apparently, the amount of repolarization of membrane potential after the depressor discharge was reduced. Both the ipsilateral and contralateral SRs stimulated individually could produce this effect (Fig.31) Importantly, this showed that events occurring at considerably later times in the flight cycle are influenced by SR input.

Elevator Motoneurons

The SR connections to depressor motoneurons differed only slightly from those seen by Burrows (1975) in *Schistocerca*. Perhaps a more important difference, however, was that neither forewing nor hindwing SRs evoked IPSPs in elevator motoneurons. A large number of elevators were sampled ($n>25$) and any possible PSPs were searched for with spike-triggered averaging (up to 512 sweeps). Possibly, in these neurons, the SRs evoked a conductance change without altering the membrane potential. However, passing tonic depolarizing or hyperpolarizing currents intracellularly did not reveal PSPs time-locked to the SR spike. Another possibility was that the presumed presynaptic inhibitory neuron was not excited to threshold by a single SR spike. However, no inhibitory response was detected with SR stimulus trains. In fact, stimulus trains almost always evoked a long latency depolarization in elevator motoneurons (Fig.32).

I have examined the response of elevator motoneurons to SR stimulation during flight. This was done by recording from an elevator muscle (m83, forewing tergosternal) while triggering SR stimulus trains from the hindwing depressor spike ($n=5$). This muscle is innervated by only one motoneuron (Tyrer and Altman 1974) so that the spikes in the EMG reflect the activity of this motoneuron. In deafferented preparations, the forewing tergosternal EMG showed between 2 and 4 spikes on each cycle (Fig.33a). The number was fairly constant in any one animal. Stimulation of the forewing SRs at 300 Hz decreased the

Figure 30. Reduction in repolarization of membrane potential in depressor motoneurons in response to bilateral forewing SR stimulation. A: Response in the first basalar motoneuron (97, upper trace) to turning on and turning off bilateral forewing SR stimulation which was timed to occur immediately following the hindwing subalar spike (SA, lower trace). The onset of each stimulus train is indicated by an arrowhead. On commencing SR stimulation, the membrane potential oscillations are superimposed on a higher level of depolarization, apparently due to a reduced repolarization of the motoneuron. On cessation of the stimulation, the amount of repolarization increases slowly. B: As in A but upper trace is an intracellular recording from from a mesothoracic forewing dorsal longitudinal motoneuron (81).

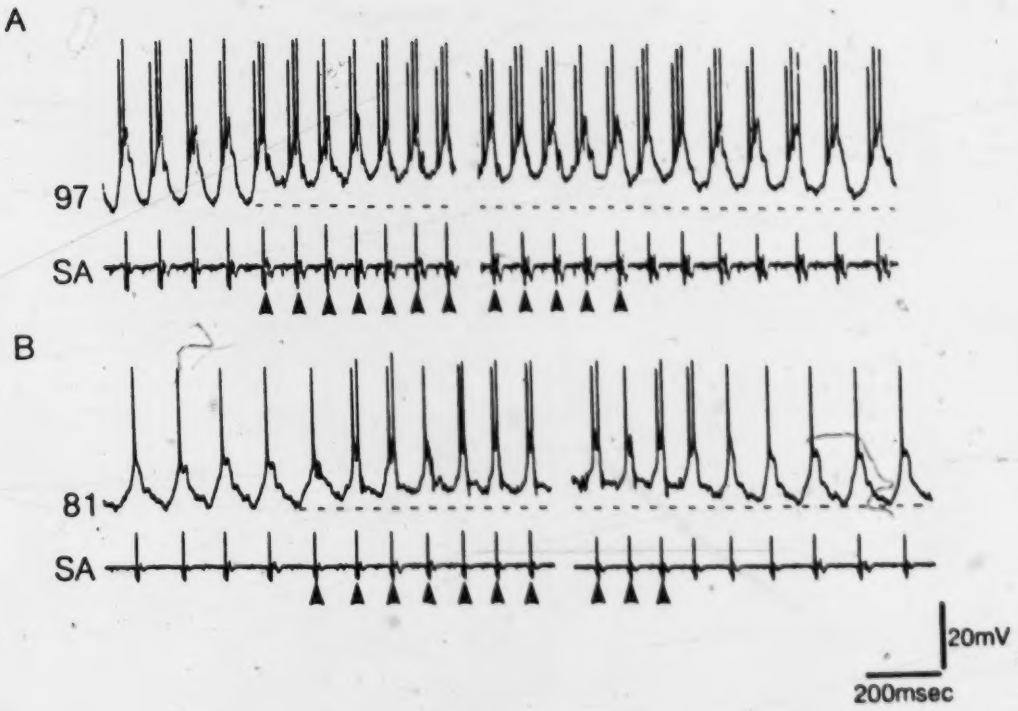

A
97
SA
B
81
SA
20mV
200msec

Figure 31. Reduction in repolarization of membrane potential in a depressor motoneuron evoked by stimulation of ipsilateral or contralateral forewing SRs. A: Response in first basalar motoneuron (97, upper trace) to triggered stimulation of the ipsilateral forewing SR (arrowhead). Lower trace: hindwing subalar (SA). B: The same neuron as in A but the contralateral forewing SR was stimulated.

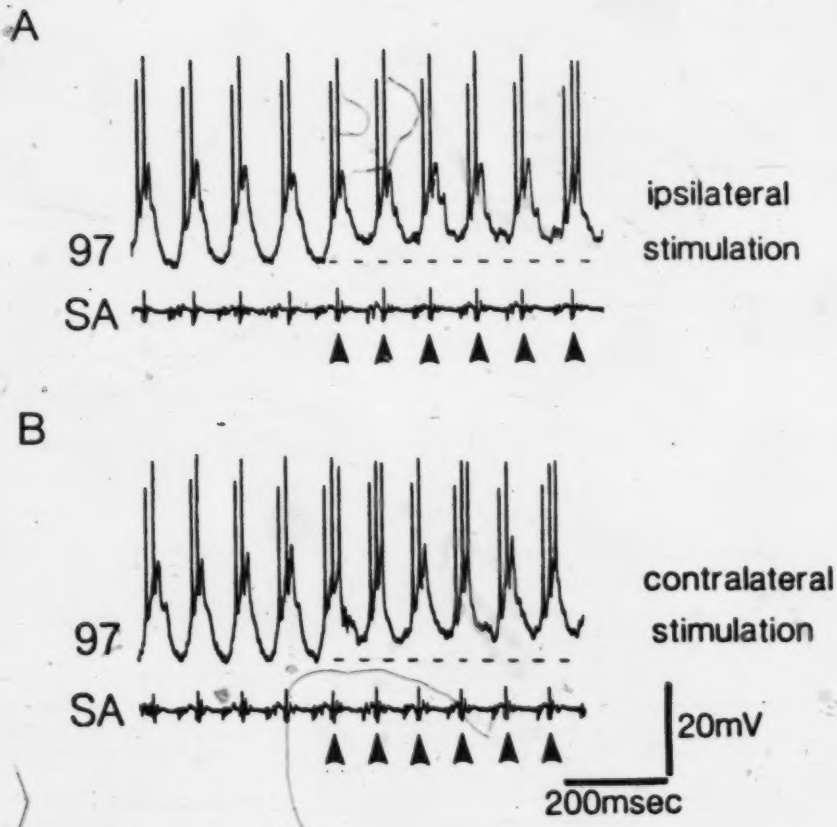

A
97
SA
ipsilateral
stimulation
B
97
SA
contralateral
stimulation
20mV
200msec

Figure 32. Trains of stimuli to the ipsilateral forewing SR evoke a long latency depolarization in elevator motoneurons. Top trace in each record is an intracellular recording from an elevator motoneuron (mesothoracic tergosternal). Bottom trace is recording from meta.n1d showing that only the SRs were being stimulated. The SRs were stimulated at meso.n1d_2. The stimulus strength was adjusted so that only the SR axon was excited. Trains were given at four different frequencies indicated above each trace. Note that in no case was a hyperpolarization evoked in the motoneuron. Each trace shows 2 - 8 superimposed sweeps.

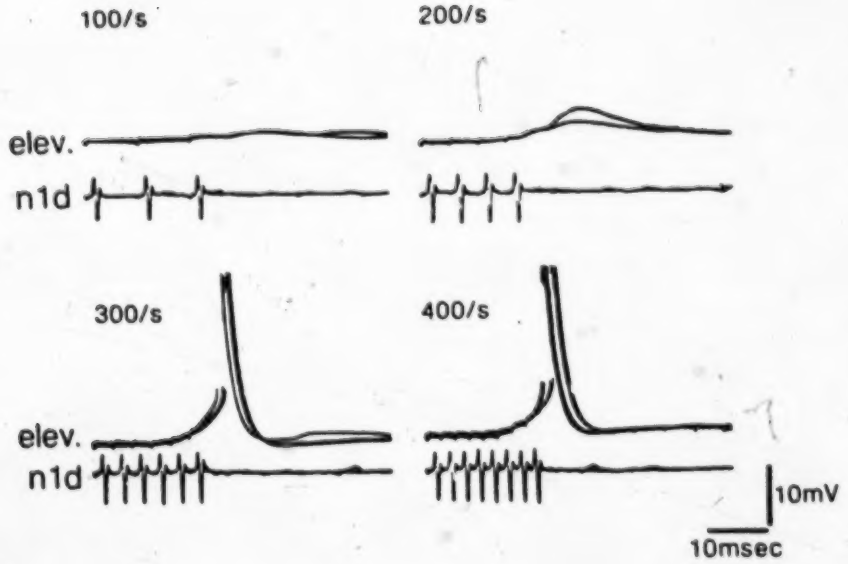
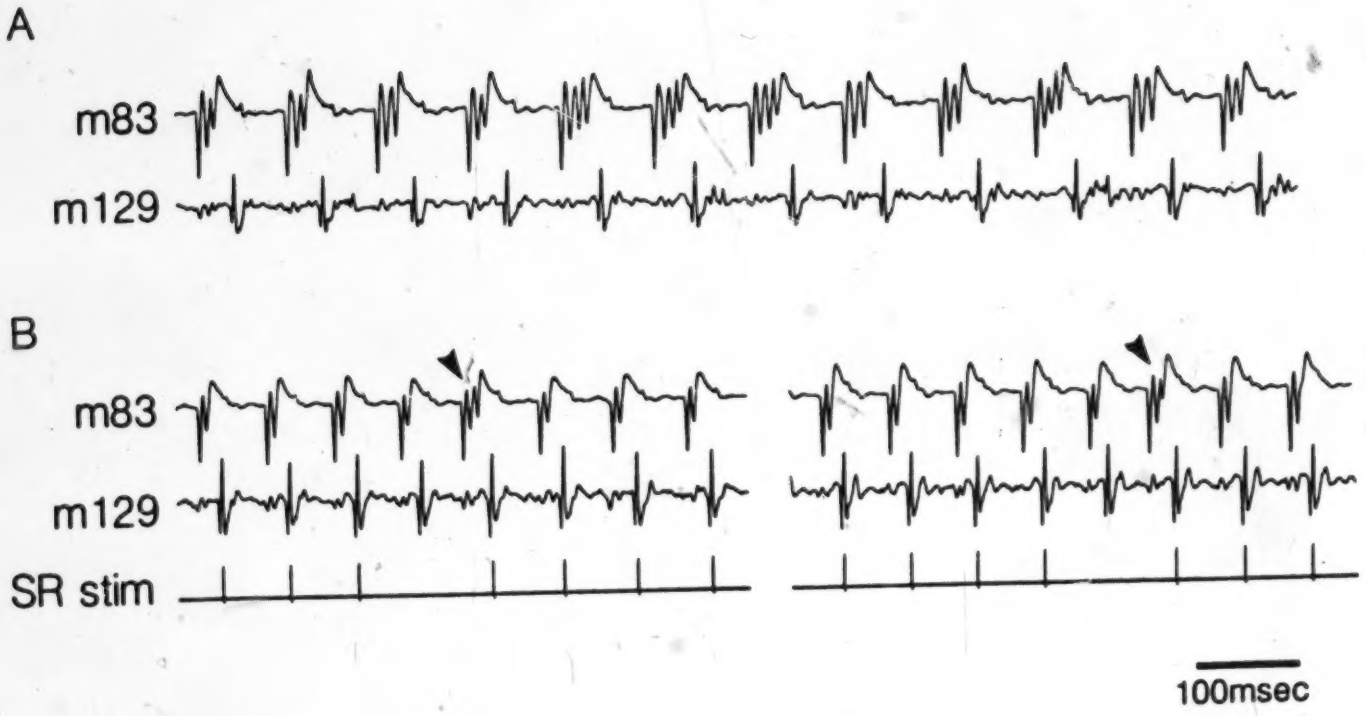

100/s
200/s
elev.
n1d
300/s
400/s
elev.
n1d
10mV
10msec

Figure 33. Inhibition of elevator activity by SR stimulation. A: Activity in the tergosternal muscle (m83, upper trace) and the hindwing subalar (m129, lower trace) during flight activity without accompanying SR stimulation. Each elevator burst contains three or four spikes. B :Activity in the same muscles when bilateral forewing SR stimulation was triggered to occur immediately following the depressor spike The onset of each stimulus train is indicated in the bottom trace. Note the reduction in the number of spikes seen in the elevator muscle so that only two spikes occurred per cycle. On some occasions the SR stimulus did not occur (indicated by the absence of the stimulus onset pulse). The following elevator EMG contained an additional spike (indicated by arrowheads). The two sets of traces in B are two different times in the same trial.


A
m83
m129
B
m83
m129
SR stim
100msec

number of spikes seen in the elevator EMG so that only one or two spikes were elicited (Fig.33b). A similar number of spikes is seen in intact animals (Wilson and Weis-Fogh 1962, Möhl 1985b). It is important to note that the elevator discharge being affected follows the SR stimulation by half a cycle. Thus, this is another example of the SRs influencing events at relatively long times after their stimulation. Furthermore this inhibition of the elevators was being exerted on each cycle. It can be seen in Figure 33 that if the SRs were not stimulated on one cycle then the elevator EMG associated with the following depressor cycle fired an additional spike. This effect could be produced by either the ipsilateral or contralateral forewing SRs when stimulated individually (Fig.34).

These results show that the SRs can inhibit the following elevator discharge. Burrows (1975) proposed that the SRs formed part of a negative feedback loop by which the amplitude of the wing movements could be regulated. On the basis of his intracellular data, he suggested that the discharge of the elevator motoneurons would excite the SRs (by raising the wing) and that the SRs would feedback to inhibit that elevator burst. However, in my preparations, SR stimulation did not evoke IPSPs in elevator motoneurons (Fig.32). In one experiment, I investigated whether SR stimulation had a short-latency inhibitory effect on an elevator motoneuron during flight activity (Fig.35). A mesothoracic tergosternal motoneuron was recorded intracellularly and a single stimulus train was applied to the ipsilateral forewing SR at different times. If the stimulation occurred during an elevator depolarization then the stimulus had no effect (Fig.35a). However, SR stimulation coinciding with the depressor discharge attenuated the following elevator depolarization (Fig.35b). Figure 35c shows the relative amount of elevator depolarization as a function of the time that the stimulus fell in the depressor cycle. There was no indication that SR stimuli coinciding with the elevator depolarization could inhibit that depolarization.

Figure 34. Elevator activity is inhibited by stimulation of either the ipsilateral or contralateral forewing SRs. Recordings from the anterior tergosternal muscle (m83, upper trace) and the hindwing subalar (SA, lower trace). A: The upper pair of traces show the elevator and depressor discharge in response to a windstream directed at the head (wind alone). The lower set of traces show the response to stimulation of the ipsilateral forewing SR. (stimulus onset indicated by pulses in bottom trace). Note the reduction in the elevator EMG to two spikes per cycle. B: As in A, but only the contralateral forewing SR was stimulated. There was a similar reduction in the number of elevator spikes as was seen for ipsilateral SR stimulation. Results in A and B were obtained from different animals.

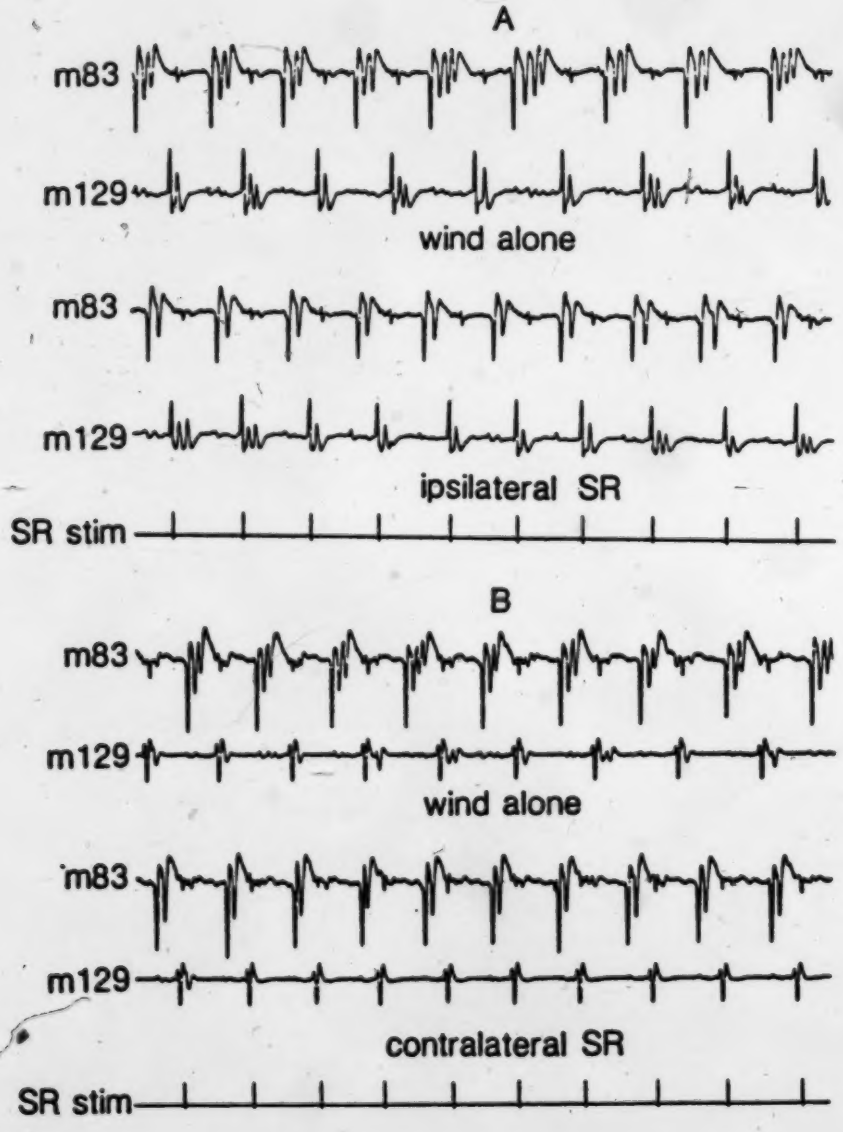
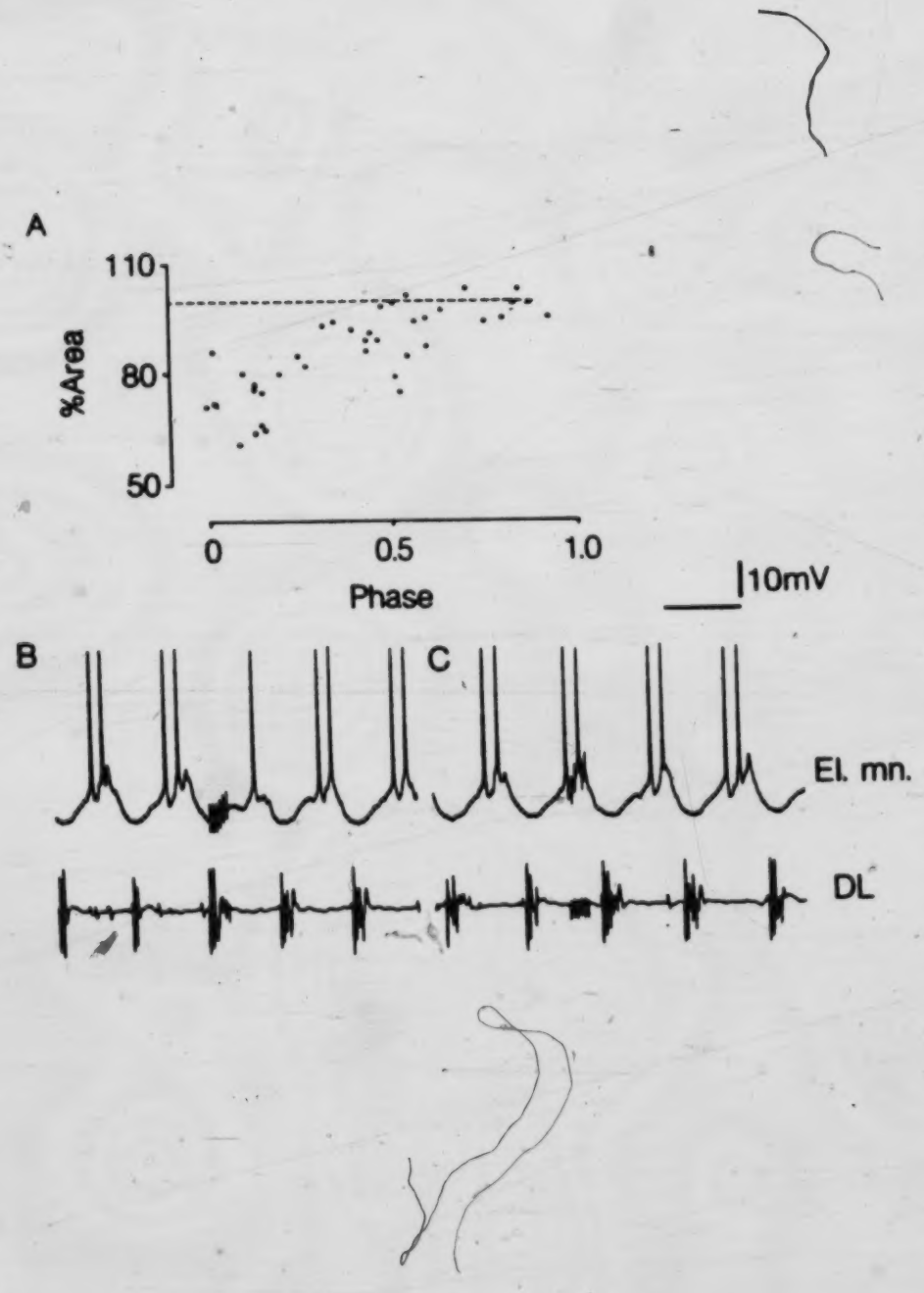

A
m83
m129
wind alone
m83
m129
ipsilateral SR
SR stim
B
m83
m129
wind alone
m83
m129
contralateral SR
SR stim

Figure 35. Inhibition of elevator motoneuron depolarization by SR stimulation. A single stimulus train was applied to the ipsilateral forewing SR afferent. Stimulus trains were presented at a low constant rate of approximately 1.5 Hz so that they fell in a wide range of phases in the oscillator cycle. A. The area of the elevator depolarized phase relative to the area of the preceding oscillator burst is plotted as a function of the phase in the depressor cycle at which the onset of the stimulus occurred. Stimuli falling before the elevator burst decreased the amplitude of the oscillation. Stimuli falling coincident with the elevator did not have any consistent effect. B. and C: Intracellular recordings of the elevator motoneuron from which the data shown in A. were calculated. In B, the SR stimulus fell within its normal range of firing in the flight cycle. The following elevator burst is substantially reduced in amplitude and fires one less spike than seen in the cycles previous and subsequent. In C, the SR stimulus falls coincident with the elevator's depolarized phase. This is not only out of the normal range of phases at which the SR burst occurs in the intact animal but has little apparent effect on the elevator motoneuron. Horizontal calibration: 100msec.


A
110
80
50
%Area
0
0.5
1.0
Phase
10mV
B
C
El. mn.
DL

Connections of SRs to Thoracic Interneurons.

Twenty-two interneurons were identified which received constant, short-latency connections from the SR afferents. Table II lists the connections each cell received from the four stretch receptors (i.e. forewing and/or hindwing, ipsilateral and/or contralateral to the soma of the interneuron). Also indicated for each interneuron is the phase of its depolarization in the oscillator cycle. The naming of the cells follows the scheme outlined by Robertson and Pearson (1983). Inspection of this table shows several points of interest. The majority of connections were to depressor interneurons (16 out of 22 cells). From the data so far, all but three interneurons received inputs from the ipsilateral SRs only (17 out of 20 cells tested). Finally, all but one interneuron of the thirteen cells examined received convergent inputs from both forewing and hindwing SRs. The one exception (neuron 507) received a bilateral forewing SR input. Three examples illustrating this convergence are shown in Figure 36. The amplitudes were generally similar from the two sources although there were some exceptions (e.g. 313, below).

Also indicated at the bottom of Table II are previously described interneurons (Robertson and Pearson 1983, 1985) which did not receive direct SR input. In the case of negative findings, the SR spike was confirmed to be conducting into the ganglion by either recording SR-evoked EPSPs in other neurons in the same hemiganglion or by intracellular recording from the SR afferent. Several of these interneurons, which do not receive SR input can reset the timing of the central rhythm and are presumed to be members of the central oscillator (cells 301, 401, 504). Only one presumed central oscillator interneuron received direct SR input (cell 501, Robertson and Pearson 1985), although this was of small amplitude (1mV) compared to the rhythmic depolarization seen in this cell during the central rhythm (ca.20mV).

Influence of SR Stimulation on Central Interneurons

TABLE II - Amplitude of Stretch Receptor evoked excitatory postsynaptic potentials (EPSPs) in locust thoracic interneurons. Entries indicate whether the cell receives ipsilateral and/or contralateral (relative to the soma) inputs from the forewing or hindwing stretch receptors. A NIL entry indicates the SR in question does not connect to the cell. No entry indicates that the connection in question has not been tested. Phase refers to the activity of the interneuron during the deafferented flight rhythm.

D : depolarization in phase with depressor motoneurons.

E : depolarization in phase with elevator motoneurons.

T : tonically active

N : not rhythmic during deafferented flight activity.

(a) Structure described in Robertson and Pearson (1983)

(b) Structure described in Pearson et al (1985).

Previously published cells which do not receive SR connections:

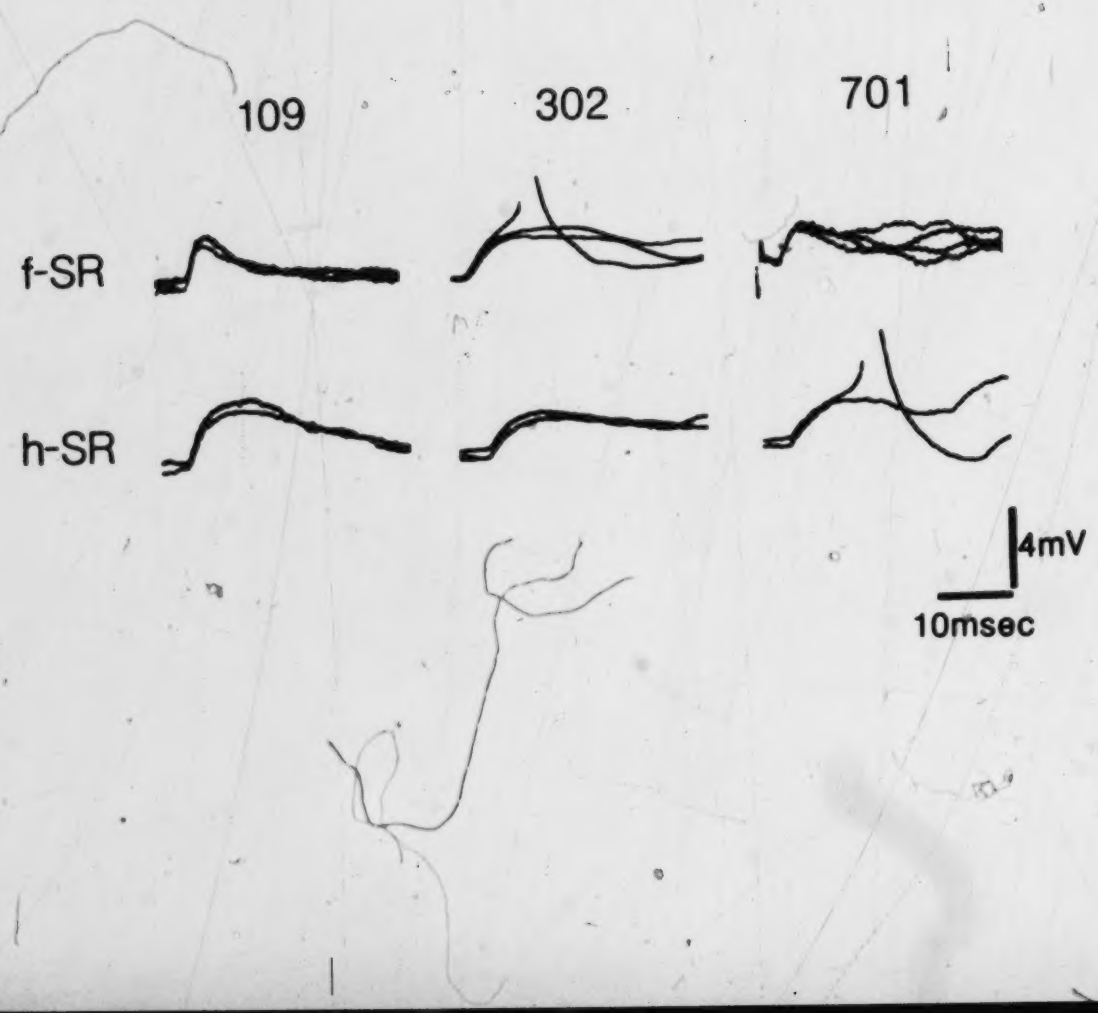
Robertson and Pearson (1983) : 201, 401

Robertson and Pearson (1985) : 206, 301, 504, 511

TABLE II

		EPSP AMPLITUDE (mV)			
		Forewing SR		Hindwing SR	
Neuron	Phase	ipsi.	contra.	ipsi.	contra.
MESO.					
109	D	2-3	NIL	2-3	NIL
115	E	1	NIL		
116	D	4	NIL		
202	D	1	NIL	1	NIL
302[a]	D	2-4	NIL	2-4	NIL
313	E	5-6	3-5	2	NIL
404[b]	T	1	NIL	0.5	NIL
507	D	2-3	2-3	NIL	NIL
603	E	4			
701[a]	D	1-2	NIL	1-2	NIL
META.					
116	D	4	NIL		
124	E	5-6		3-4	
208	D	2-5	NIL	2	NIL
318	D	1-2	NIL	1-2	NIL
323	D			2-3	NIL
501[a]	D	1	NIL	1	NIL
503[a]	D	2	NIL	2	NIL
505	D	2	NIL		
506	D	2	2		
519	N	5-6	NIL		
520	D	1	NIL	1	NIL
521	D	1-2	NIL		

Figure 36. Convergent input to flight interneurons from the forewing and hindwing SRs. Three examples of EPSPs are shown. Top row: EPSPs evoked from the forewing SR (f-SR) in each of three mesothoracic interneurons. Bottom row: EPSPs from the hindwing SR (h-SR) in these neurons. Each record shows 2 to 3 superimposed sweeps from the indicated ipsilateral SR. The amplitude are similar from the two SRs. Each recording was from a different animal.


109
302
701
f-SR
h-SR
4mV
10msec

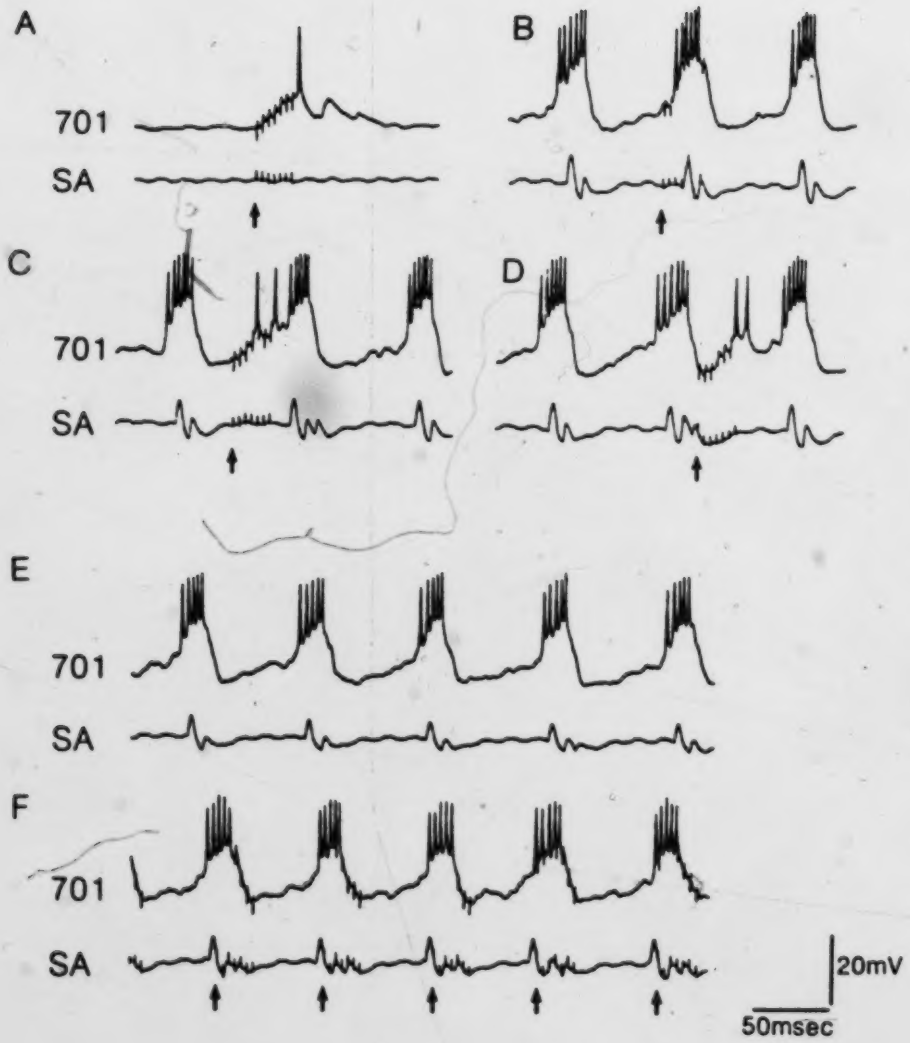
Depressor Interneurons

The majority of SR connections were to interneurons depolarized in phase with depressor motoneurons. Of interest was what effect SR input had on the activity of these interneurons.

Figure 37 shows an example where SR stimulation had no strong effects on the discharge of the neuron. This cell (701; n=18) has been previously described (Robertson and Pearson 1982, 1985). It is an excitatory premotor interneuron to both forewing and hindwing depressor motoneurons (Robertson and Pearson 1985). In the quiescent state, a train of stimuli to the f-SR axon caused a compound EPSP of approximately 10mV in 701 (Fig.37a). Single stimulus trains were presented every 6 -10 cycles of the deafferented rhythm (Fig.37 b,c,d). If the stimulus fell between the depressor spikes, phases at which SR input would not normally occur, the stimulus produced extra spikes in 701's membrane potential oscillations (Fig.37c,d). On the other hand, if stimuli occurred in the range of natural SR phases, then there was no change in either spike frequency or the number of spikes (Fig.37b). Similarly, if the stimulus was triggered to occur on every cycle at a time close to that which occurs in the intact animal (Fig.37f), the number of spikes and the spike frequency were the same as seen without stimulation (Fig.37e). The failure of SR stimulation to alter 701's discharge characteristics at normal phases appeared to lie in the relatively low amplitude of its input but more importantly in the fact that it was already strongly depolarized when the SR input reached the cell. Another neuron which falls into this category is the rhythm generating interneuron 501 (Robertson and Pearson 1985; n=6). Triggered stimulation of the wing SRs during flight did not evoke any strong influences on this neuron.

Also of interest was the fact that responses to the stimulation could be seen even when SR input occurred at inappropriate phases in the flight cycle (e.g. Figs.37c and 37d; see also examples below). This suggests that the forewing SRs are not presynaptically gated by the rhythm generator during the elevator phase. Altman and coworkers (Altman et al

Figure 37. Effect of ipsilateral forewing SR stimulation on the rhythmic activity of a depressor interneuron (701). In each record the upper trace is an intracellular recording from 701, the lower trace is the EMG recorded from the hindwing subalar muscle (SA). A : Compound EPSP elicited in 701 in non-rhythmic state. B,C,D : Single trains of stimuli were given during the deafferented rhythm. B : the stimulus falls close to the natural phase at which SR bursts occur. Note that neither the number of spikes nor the spike frequency is altered from that which is generated by the central oscillator. C,D : Stimuli fall in inappropriate phases of the flight cycle. Extra spikes are elicited, indicating that the failure of the stimulus given in (B) is due to the already depolarized state of the cell. E : The rhythmic activity in 701 when driven by the central oscillator with no SR stimulation. F : Trains of stimuli are triggered to occur on every flight cycle of the central oscillator rhythm. In this and all subsequent figures, the time of onset of the f-SR stimulus train is indicated by arrows underneath the record.


A
701
SA
B
C
701
SA
D
E
701
SA
F
701
SA
20mV
50msec

1980, Peters et al 1985) found that the hindwing SR afferents receive synaptic boutons in electron micrographs. It is not known if the f-SR also receives synaptic input nor whether these boutons are inhibitory. If the central oscillator does presynaptically inhibit the SR afferents (and I have found no evidence to suggest this), it is not distributed to all SR afferent terminals.

The SRs evoked strong effects in depressor interneurons which displayed weaker membrane potential oscillations. An example was cell 109 (n=8). In this cell, stimulating the SRs in the quiescent state produced a depolarization as large as that seen during the deafferented rhythm (Fig.38a). If the SR input occurred either slightly before or slightly after the centrally derived depolarization then the duration of the depolarization was prolonged (Fig.38c). In some cases, neurons which received EPSPs from the SRs showed subthreshold oscillations in phase with depressors (neurons 109, 505, 506). Typically, these cells would fire action potentials for the first 12 - 20 cycles, after which only subthreshold oscillations occurred. An example of this is neuron 506 (Fig. 39; n=4). Neuron 506 has a cell body located in the ventrolateral part of the metathoracic ganglion. The neurite from the cell body projects anteriorly and dorsally. Slightly lateral to the midline there is a short, thick process from which arises much of 506's arborization in the dorsal neuropil. The axon projects from this process at first directed laterally and posteriorly. On the contralateral side of the ganglion, the axon makes a sharp turn to project anteriorly in the mesothoracic-metathoracic connective. The influences of SR stimulation on 506's activity are shown in Figure 39c. The central rhythm generator evokes weak membrane potential oscillations in phase with depressors, which were often subthreshold. When the SRs were triggered to occur on every cycle, these rhythmic depolarizations could be forced above threshold, so that two or three spikes were elicited on each cycle. For neurons such as 506, SR input appears to be important for determining the actual time when they spike. However, the effects on these neurons is largely confined to the duration of the SR stimulus. Figure 40 shows a depressor interneuron (208, n=4) where the effects of SR

Figure 38. Effect of forewing SR stimulation on a depressor interneuron (109). Upper records : intracellular recording from cell 109. Lower traces : EMG recordings from the hindwing dorsal longitudinal muscle (DLM). The large vertical transients on the intracellular record are stimulus artifacts. A : Compound EPSP elicited in 109 in non-rhythmic state. B,C,D : Single stimulus trains given during the deafferented rhythm (onset indicated by arrows) B : The stimulus falls coincident with the 109 depolarization, causing only a slight increase in the level of depolarization. C : The train of stimuli applied slightly later than in (B) Note that the 109 depolarization is prolonged for the duration of the stimulus. D : Train of stimuli applied midway between depressor spikes. The SR-evoked depolarization is still expressed even though an inappropriate phase for the SR burst.

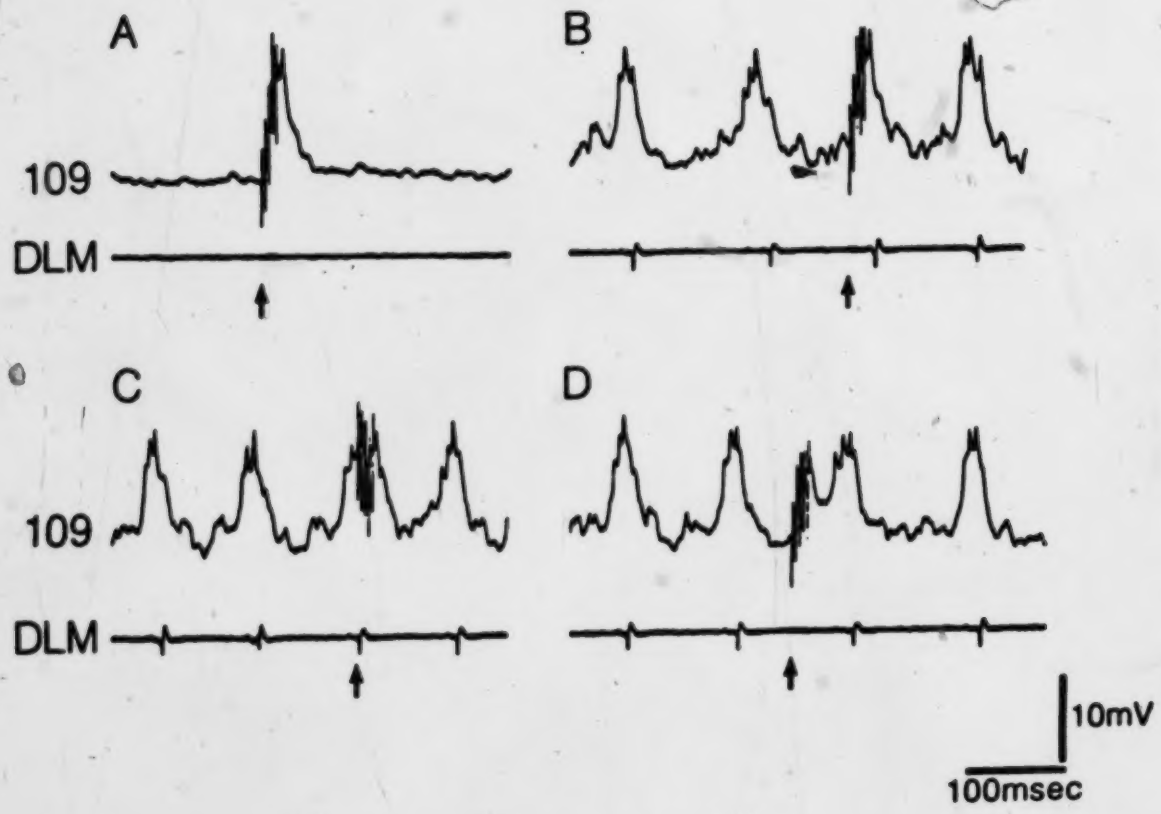
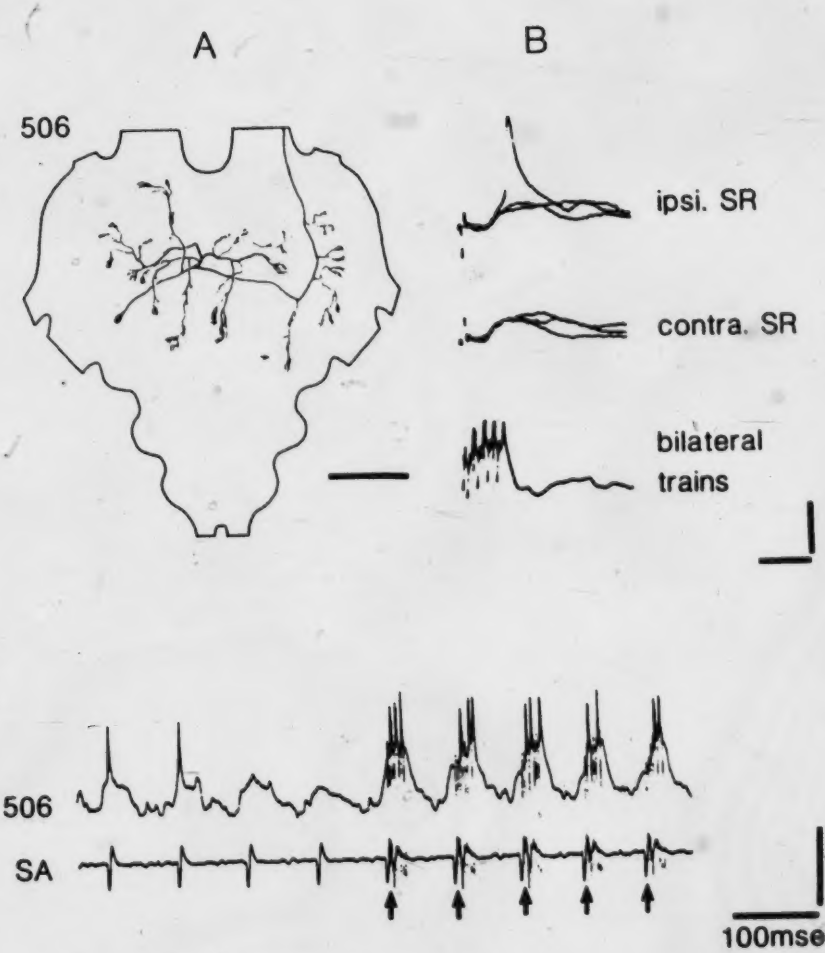

A
B
109
DLM
C
D
109
DLM
10mV
100msec

Figure 39. Stretch receptor connection to an interneuron with subthreshold rhythmic oscillations (506). A: Structure of neuron 506. Calibration for this and all subsequent neuron drawings: 200μm. B: Upper and middle panels: EPSPs in 506 from the ipsilateral and contralateral SRs, evoked by stimulation of pro.n6. Vertical calibration: 4mV. Horizontal calibration: 10msec. Middle panel: response in 506 to a train of stimuli applied bilaterally to pro.n6. Vertical calibration: 10mV. Horizontal calibration: 40msec. Note the short-latency burst followed by a hyperpolarization. C : Rhythmic membrane potential oscillations in 506 before and during bilateral stimulation of the SRs on every cycle. Before introducing the stimulation, the neuron fired a single or no spikes on top of a small rhythmic depolarization in phase with depressors. When stimulation was commenced, these oscillations were forced above threshold so that 2 - 3 spikes occurred on each cycle.


A
B
506
LF
ipsi. SR
contra. SR
bilateral
trains
506
SA
10mV
100msec

stimulation were not confined to the duration of the stimulus. Neuron 208 has a medial cell body from which arises a neurite projecting anteriorly and dorsally. In the dorsal neuropil, the main neurite forms a characteristic looping structure, directed laterally. From the loop the axon descends to the abdominal connective, ipsilateral to cell body. The major neuropil branching occurs in the anterior portion of the metathoracic ganglion. Cell 208 receives EPSPs from the ipsilateral forewing and hindwing SRs (Fig.40). No contralateral inputs have been found. Figure 41 shows the effects of stimulating the SRs from the hindwing depressor spike. The stimulus trains in this case were of approximately 18 msec duration. Upon introducing stimulation, there was a marked prolongation of the duration of its bursts which were imposed on a high level of tonic depolarization. The normal inhibition which occurred during elevator phase was strongly attenuated. Its former pattern of activity returned upon removing the stimulation. This cell was clearly being very powerfully modulated by SR input.

Elevator Interneurons

An unexpected finding was that not only neurons depolarized in phase with depressors receive SR input. Three neurons depolarized in the elevator phase have been identified which receive strong (>4mV) EPSPs from the SRs (neurons 124, 313, 603). Only one of these (cell 313) has been examined for its response to SR stimulation during flight activity. The structure of this cell is shown in Figure 42a. Its characteristic morphological features were a midline cell body with a looping process joining the main transverse neurite. This crossed the ganglion giving rise to a descending axon which travelled medially in the meso - meta connective. Arising from the main neurite were 2 branches which projected anteriorly in an approximate line with the inside edge of the prothoracic connectives. It showed some variability in its secondary branching pattern but had a very consistent physiology (n=6).

An action potential was elicited for every spike from the ipsilateral f-SR afferent. The ipsilateral f-SR always produced spikes in 313. The contralateral f-SR evoked a

Figure 40. SR input to a metathoracic depressor interneuron (208). A: Structure of 208 in the metathoracic ganglion. B: EPSPs in 208 evoked by the forewing and hindwing SR afferents. Each recorded from a different animal. In both cases, the cell was hyperpolarized to prevent tonic spiking. Note the different gains for these two traces. EPSP from the forewing SR was approximately 5mV while the input from the hindwing SR was approximately 2mV.

A
208
B
f-SR
h-SR
8mV
4mV
10msec

Figure 41. Modification of activity in 208 by bilateral SR stimulation. Upper trace: intracellular recording from 208. Lower trace: hindwing subalar (SA). Without SR stimulation, the 208s showed rhythmic depolarizations in phase with depressors. When SR stimulation was commenced (indicated by arrows beneath the EMG record), there was a dramatic increase in the baseline level of depolarization in 208. The amplitude of the oscillations were markedly reduced and extra spikes occurred between the depressor bursts. The rhythmic activity rapidly returned to its prestimulation levels after cessation of the stimulation.

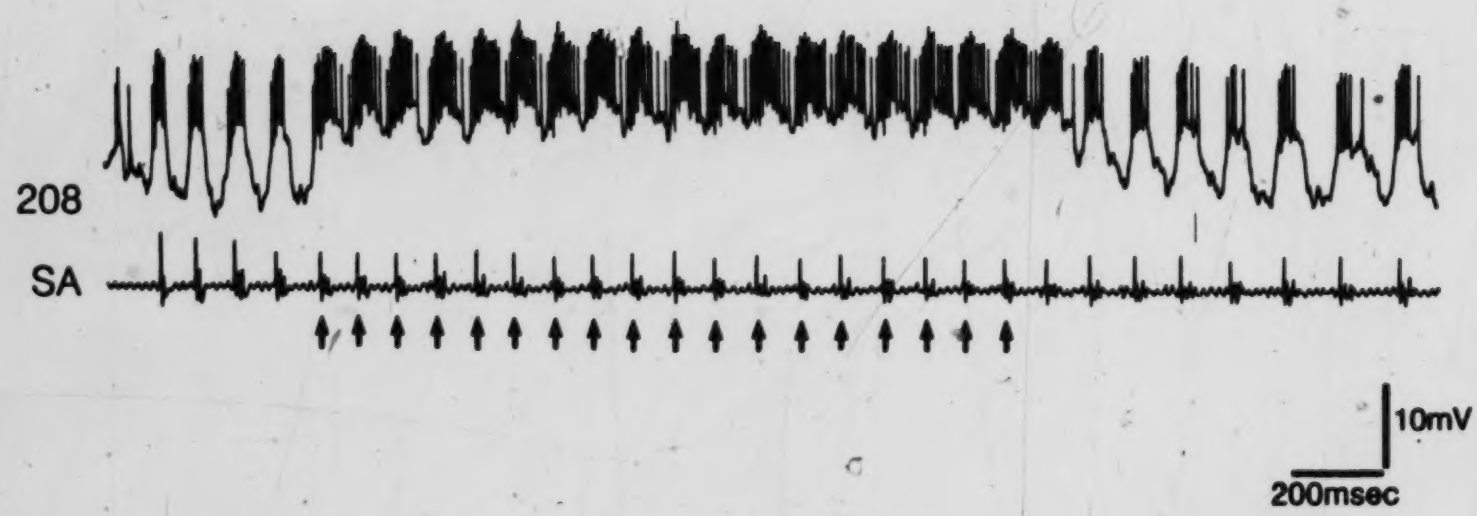
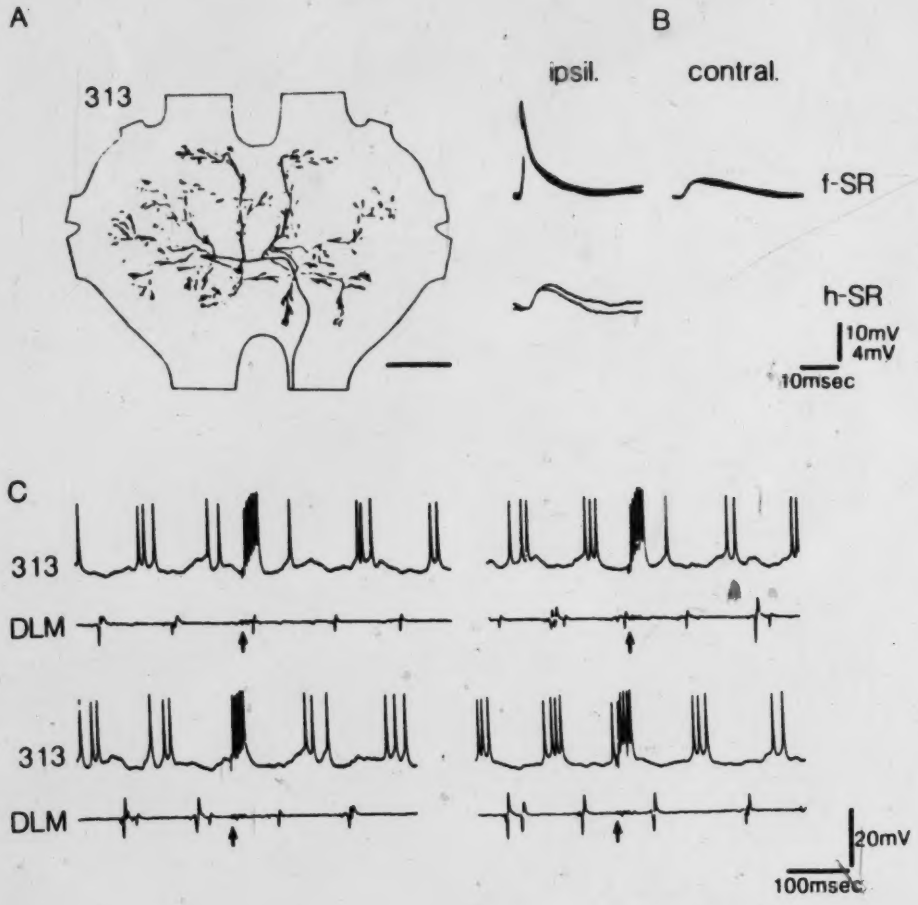

208
SA
10mV
200msec

Figure 42. SR input to a mesothoracic elevator interneuron (313). A : Structure of 313 in the mesothoracic ganglion. B : EPSPs evoked in 313 from the SRs. Each spike in the ipsilateral f-SR evokes a single action potential in 313. The contralateral f-SR evokes only slightly weaker EPSP judged by the slower rise-time. Spikes were not always seen on top of the EPSP from this f-SR in every animal, although the underlying EPSP was always at least 6mV. The h-SR gives a much weaker input (note different calibration scale) and only from the ipsilateral side. C : Effects of a single stimulus train applied to the ipsilateral f-SR at different times in the flight rhythm. The normal phasing of this neuron is a weak depolarization occurring midway between depressor muscle spikes. SR stimulation produces a similar discharge in 313 irrespective of when that input occurs in the flight cycle.


A
313
B
ipsil.
contral.
f-SR
h-SR
10mV
4mV
10msec
C
313
DLM
313
DLM
20mV
100msec

smaller but still large EPSP. There was a difference between the forewing and hindwing SR input. Cell 313 received an input only from the ipsilateral h-SR (Fig.42b) and this was much weaker than the response to f-SR. A train of stimuli applied to the ipsilateral f-SR produced a burst of spikes in 313. The f-SR stimulation was found to elicit a burst of spikes in 313 independently of the time in the cycle at which that stimulus occurred (Fig.42). If appropriately timed to where the SR burst would normally occur then this cell in fact discharged primarily in phase with depressors (Fig.43). However, the centrally derived elevator spikes were still evident between the stronger bursts elicited by SR stimulation.

A more strongly rhythmic elevator interneuron (cell 124, Fig.44) also received large amplitude EPSPs from both f-SR and h-SR (n=3). This neuron discharged early in the elevator phase. Its response to repetitive SR stimulation was not examined. However, one would expect that SR stimulation at the time of the depressors would advance the onset of firing in this neuron

Another non-depressor interneuron (cell 308) was also powerfully modulated by the SRs but in this case by an inhibitory input. The structure of this cell is shown in Figure 45. It has a lateral cell body with a contralaterally descending axon which projects posteriorly into the abdominal connectives. Numerous fine branches project medially from this axon in the metathoracic and fused abdominal ganglia. From the transverse neurite in the centre of the ganglion are two sets of anteriorly directed branches which project almost to the ganglion's anterior border.

Cell 308 differed from the previously described neurons in that it received an inhibitory postsynaptic potential (IPSP) from all four SRs. It is the only interneuron I have found to respond with an IPSP for every afferent spike. The longer latency (approximately 8 msec) and its opposite polarity suggested these connections are via at least disynaptic pathways. However, the IPSPs occurred for every SR spike and were found in all animals where this cell was recorded (n=10). Of additional interest is the fact

Figure 43. Modification of activity in 313 by SR stimulation. A: Rhythmic activity of a 313 interneuron (upper trace) in response to wind on the head. Note the hyperpolarized phase of this neuron coincided with the hindwing depressor EMG (SA, lower trace). B: The same neuron as in A but in this case, both forewing SRs were stimulated during flight (indicated by arrows). The normal rhythmic hyperpolarization in depressor phase was now replaced by a high frequency burst of spikes. Note also that spikes were still seen to occur during the elevator phase.

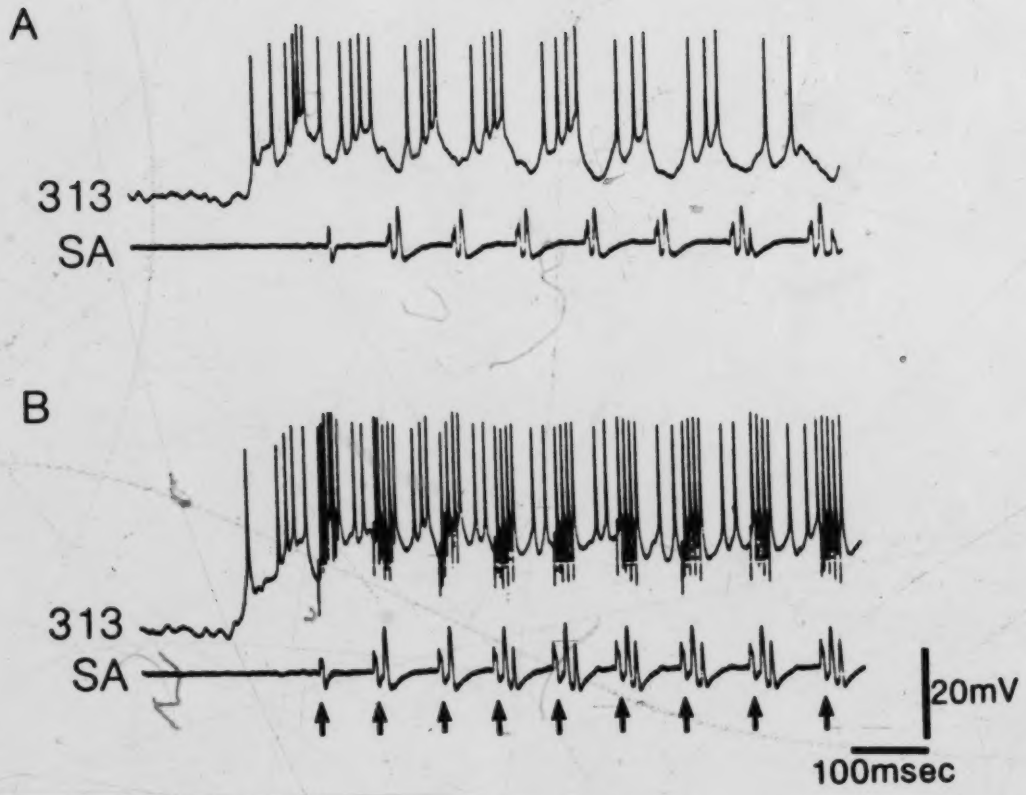

A
313
SA
B
313
SA
20mV
100msec

Figure 44. Stretch receptor input to a metathoracic elevator interneuron (124). A : Structure of I24 in the metathoracic ganglion. B : EPSPs evoked in 124 from the ipsilateral forewing and hindwing SRs. The recordings are from different animals. The ipsilateral f-SR evokes a single spike followed by a second delayed excitation with spikes occurring at variable latency. The ipsilateral h-SR also gives rise to an EPSP and spikes but without the delayed excitatory component. C : The deafferented activity of 124 consists of a burst immediately following depressor activity and a phasic hyperpolarization preceding the subalar (SA) muscle spike.

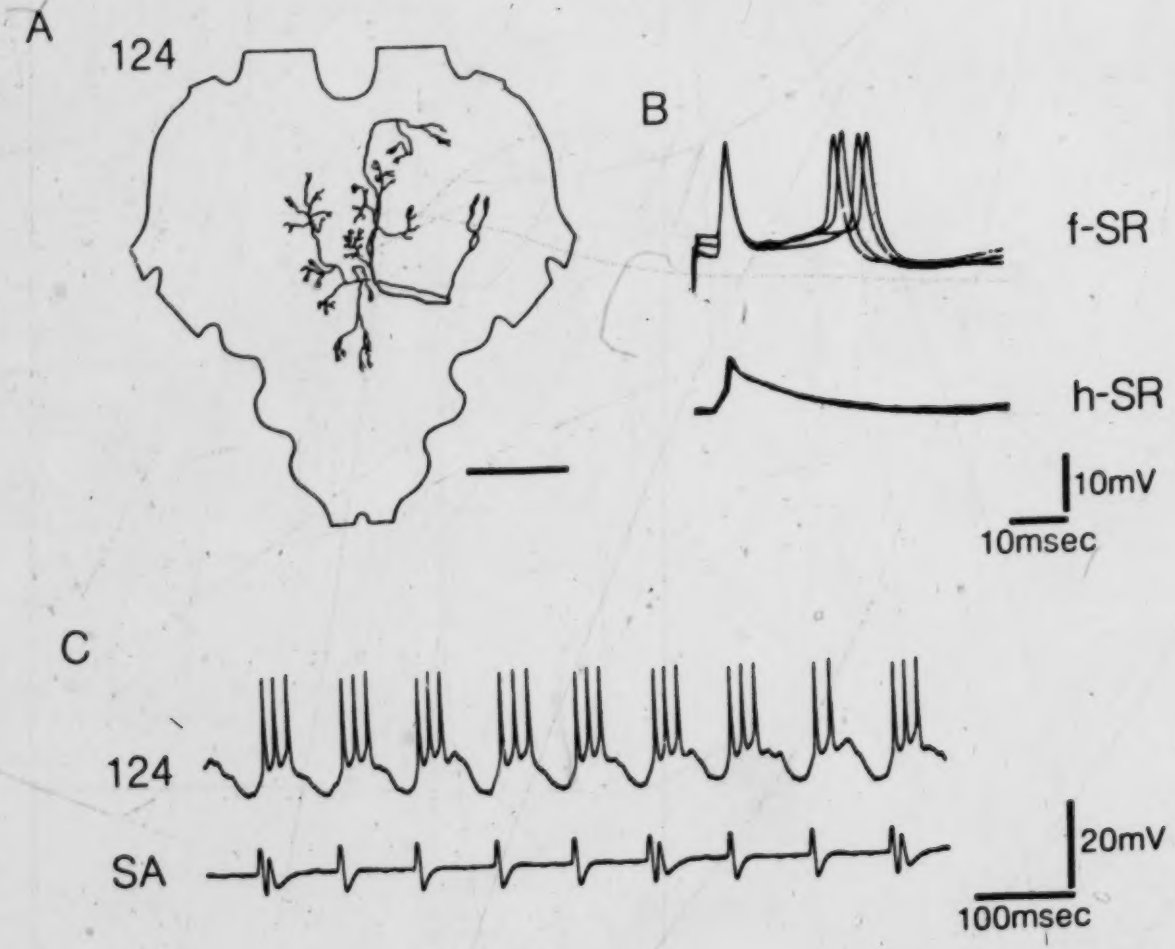
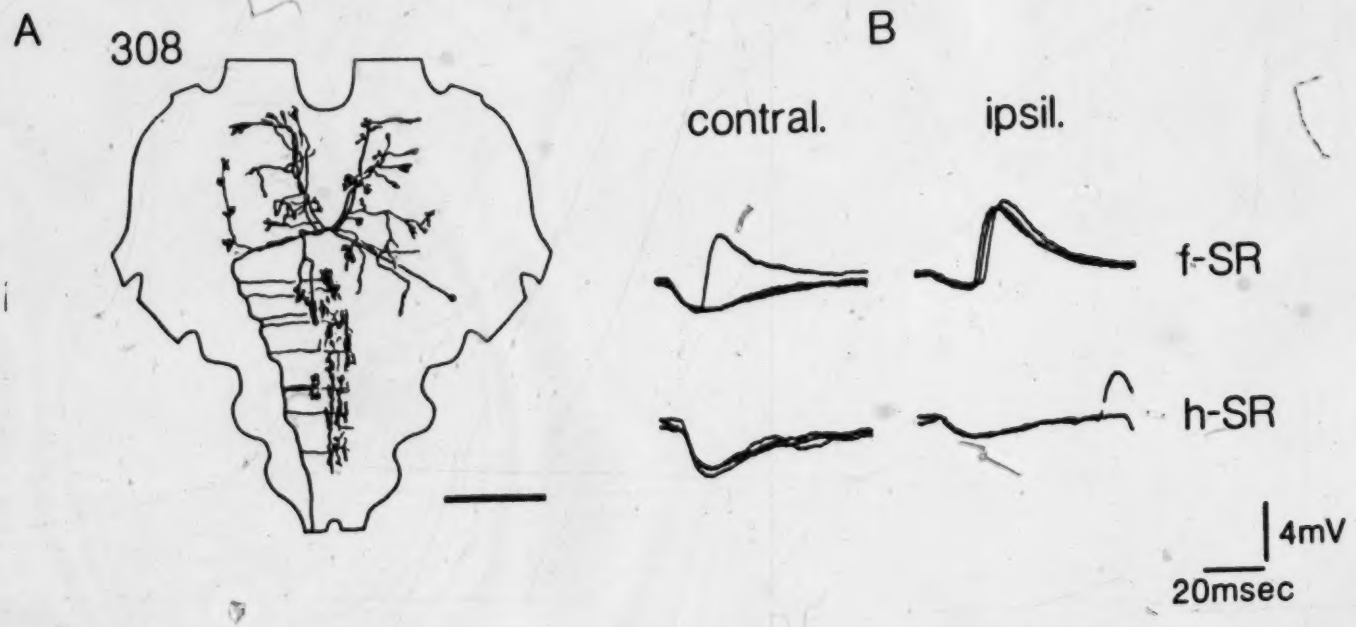

A
124
B
f-SR
h-SR
10mV
10msec
C
124
SA
20mV
100msec

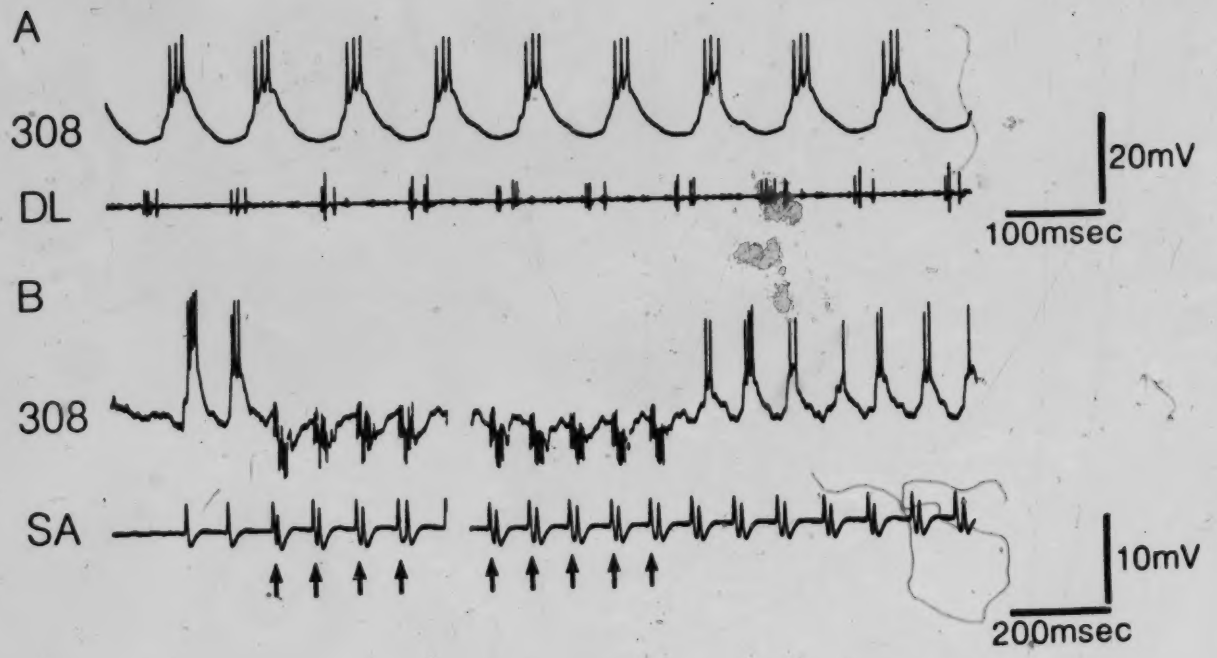
Figure 45. A metathoracic interneuron (308) receiving inhibitory postsynaptic potentials (IPSPs) from the SRs. A : Structure of cell 308 in the metathoracic ganglion. B : PSP's evoked in 308 from the SRs. The ipsilateral f-SR evokes a dual component PSP, consisting of an initial IPSP terminated by a rapidly rising EPSP. The contralateral f-SR usually gives only an IPSP but this is sometimes terminated by a rapidly rising EPSP. Both hindwing SRs evoke only an IPSP. The amplitude of the ipsilateral IPSPs is smaller than that of the contralateral IPSPs for both wing SRs. The hindwing and forewing PSP's were recorded from different animals but each segmental pair was recorded in the same animal. C : Effects of stimulus trains to one or other or both forewing SRs. Results from the same animal shown in B. Stimulating the contralateral f-SR causes a longer duration IPSP. The delayed excitatory EPSPs can be seen to be shunted by the preceding IPSP. Stimulating the ipsilateral f-SR delays the excitatory component until the end of the stimulation. Stimulating both f-SRs together results in an IPSP which repolarizes more rapidly than would occur without the delayed excitatory component. However, the depolarization evoked by the ipsilateral f-SR is shunted by the inhibition from the contralateral f-SR.


A
308
B
contral.
ipsil.
f-SR
h-SR
4mV
20msec
C
contralat.
ipsilat.
bilateral

that the ipsilateral f-SR gave rise to a dual response. This consisted of an IPSP terminated by a rapidly rising EPSP (Fig.45b). This dual component PSP was always seen from this SR. The PSPs do not appear to be via a multicomponent synapse. Firstly, while the IPSP occurred with a surprisingly constant latency, the delayed excitatory PSP did show variability. Secondly, the IPSP evoked from the contralateral f-SR was sometimes, but never always, terminated by a similar EPSP suggesting an all-or-nothing event was responsible (Fig.45b). The EPSP also did not appear to be a response to the preceding hyperpolarization, since the h-SRs gave rise to similar amplitude IPSPs but never to the longer latency EPSP. Thus the occurrence of the excitatory component appeared to be via a parallel excitatory synaptic pathway. There was also a difference in the inhibitory input from the two sides with the IPSP from the contralateral forewing and hindwing SRs being of greater amplitude than that from the ipsilateral SRs. These differences in input from the two sides resulted in quite different responses in 308 to trains of stimuli from the forewing SRs (Fig.45c). Stimulus trains applied to the contralateral f-SR evoked a long-lasting IPSP with a delayed excitatory component now more apparent. On the other hand stimulating only the ipsilateral f-SR produced a delayed excitatory response but the time to the peak of this response was delayed by the preceding IPSPs (cf Fig.45b,top right). If both f-SRs were stimulated simultaneously then the hyperpolarization evoked from the contralateral EPSPs shunted the depolarization evoked by the ipsilateral f-SRs. The overall result was of an IPSP which rapidly repolarized to resting membrane potential levels.

The strength of this inhibitory input was clearly seen if stimulation was presented during the flight rhythm. The deafferented activity of this cell showed that the SR input was similar to the synaptic events imposed by the central rhythm generator at the depressor phase (Fig.46a). Most notable was an IPSP immediately preceding the depressor EMG with a depolarization after the occurrence of the depressor bursts. The depolarization always occurred immediately following the depressor activity. The recordings obtained in intact animals by Möhl (1985b) show that the forewing SRs always discharge beyond the

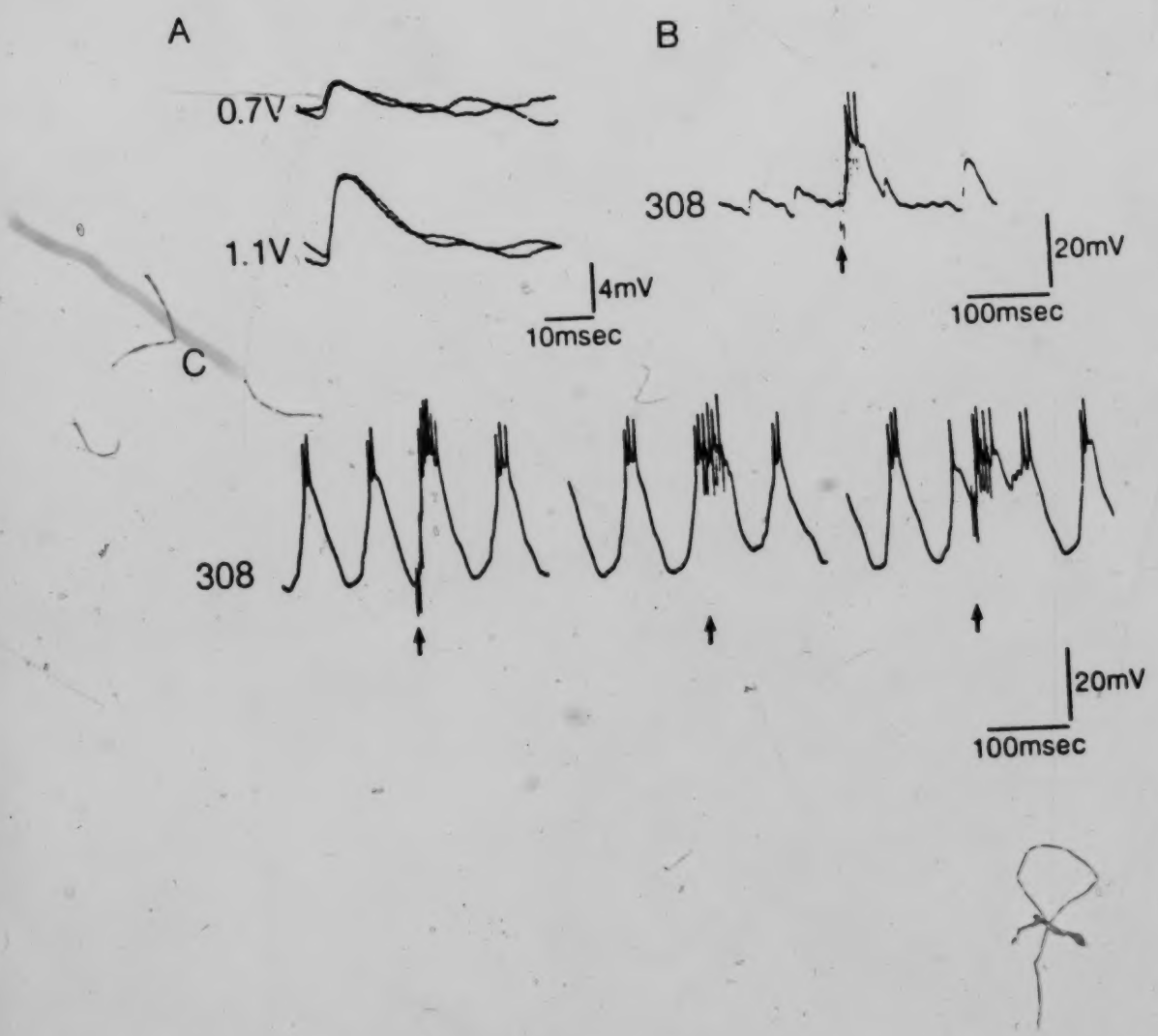
Figure 46. Abolition of rhythmic depolarizations in 308 by stimulation of the SRs. A: The activity seen in 308 during the deafferented rhythm. Upper trace : 308, lower trace : dorsal longitudinal motoneuron spikes (DL) recorded at meta.nld. There is a hyperpolarization preceding depressor activity with a depolarization occurring soon after the termination of the depressor discharge. B : Introducing bilateral stimulation of the f-SR afferents immediately following the depressor spike can completely inhibit the centrally derived depolarizations. Arrows indicate the time of SR afferent stimulation. Depolarizations reappear on terminating SR stimulation. A section of the record between the beginning and end of stimulation has been deleted.


A
308
DL
20mV
100msec
B
308
SA
10mV
200msec

hindwing depressor spike (because the hindwings lead the forewings). Mimicking this by triggering f-SR stimulation to occur immediately following the depressor spike caused the rhythmic depolarization seen in cell 308 to be completely abolished (Fig.46b). The rhythmic depolarizations re-appeared on the next cycle following cessation of stimulation. While in the intact animal such delayed bursts may not occur on every cycle, the essential point is that the centrally derived depolarization was inhibited by the stimulus and that some inhibition would be expected to always occur on every cycle in an intact animal.

The question naturally arose as to whether this neuron might be functionally inactive in the intact animal. If this were so then there seemed little advantage in the rapid repolarization evoked by the ipsilateral f-SR. The answer to this may be that the rapid repolarization enables inputs from other wing afferents to depolarize the neuron. Two groups of flight related afferents travel in n1c (the tegula and campaniform sensilla). In one animal, I tested whether these afferents might connect to 308. Cell 308 was powerfully excited with a short latency (5 msec) by electrical stimulation of the mesothoracic n1c (Fig.47a). The actual receptors responsible are unknown and this stimulation is probably not physiological. However, the short latency together with the fact that this excitatory input was not gated (Fig.47c) strongly suggested a direct excitatory action from these receptors to 308. These observations suggest the activity of 308 in an intact animal would be derived from at least three sources. The central oscillator depolarizes this neuron immediately after the depressor spike. The inhibitory input from both forewing and hindwing SRs shunts at least part of this central depolarization but the amount will depend on the time at which forewing SR activity ends. The delayed excitatory component of the f-SR rapidly repolarizes the membrane potential which enables other, as yet unidentified afferents, to further depolarize the cell. Both the tegulae and the campaniform sensilla, whose axons travel in n1c, are active during the downstroke (Gettrup 1966, Neumann 1985).

Figure 47. Cell 308 receives excitatory input from non-SR wing afferents. A : EPSPs from the contralateral meso.n1c occur with short latency and are graded with stimulus intensity. Threshold was 0.7V. Increasing the voltage to 1.1V evokes an EPSP of approximately three times the amplitude. B : A train of stimuli (25 msec, 250/s) bilaterally to meso.n1c evokes a large depolarization in 308. C : Both meso.n1c's were stimulated with a train of pulses during flight. Onset of the stimulation is indicated by arrows. The figure shows stimuli falling at three different times in the flight cycle. Note that a strong excitatory effect was apparent in all three examples shown suggesting a direct excitatory effect from the sensory afferents to 308.


A
0.7V
1.1V
4mV
10msec
B
308
20mV
100msec
C
308
20mV
100msec

A surprising finding was that not only rhythmically active interneurons received SR input. I have found a neuron (519, Fig.48), which did not receive rhythmic input during central oscillator activity. This cell is a metathoracic interneuron with a medial cell body. The axon arises from the main neurite to ascend anteriorly in the contralateral meso-meta connective. A finer process descends posteriorly. Ipsilateral to the cell body is a thick process which ascends anteriorly from the main neurite. This cell was not phasically active during the deafferented rhythm (Fig.48c). In recordings to date (3 animals), 519 always exhibited spontaneous activity. Whether this reflected injury due to the electrode penetration or was a physiological property of the cell is not known. This cell received a large amplitude EPSP from f-SR (Fig.48b). The hindwing SR input has not been examined. Triggering forewing SR input to occur on every cycle elicited a rhythmic depolarization in phase with the depressors (Fig.48d). The spontaneously occurring spikes between the depolarizations were still evident. As was found for neuron 313, similar bursts could be elicited irrespective of the time in the cycle at which the input occurred (not shown).

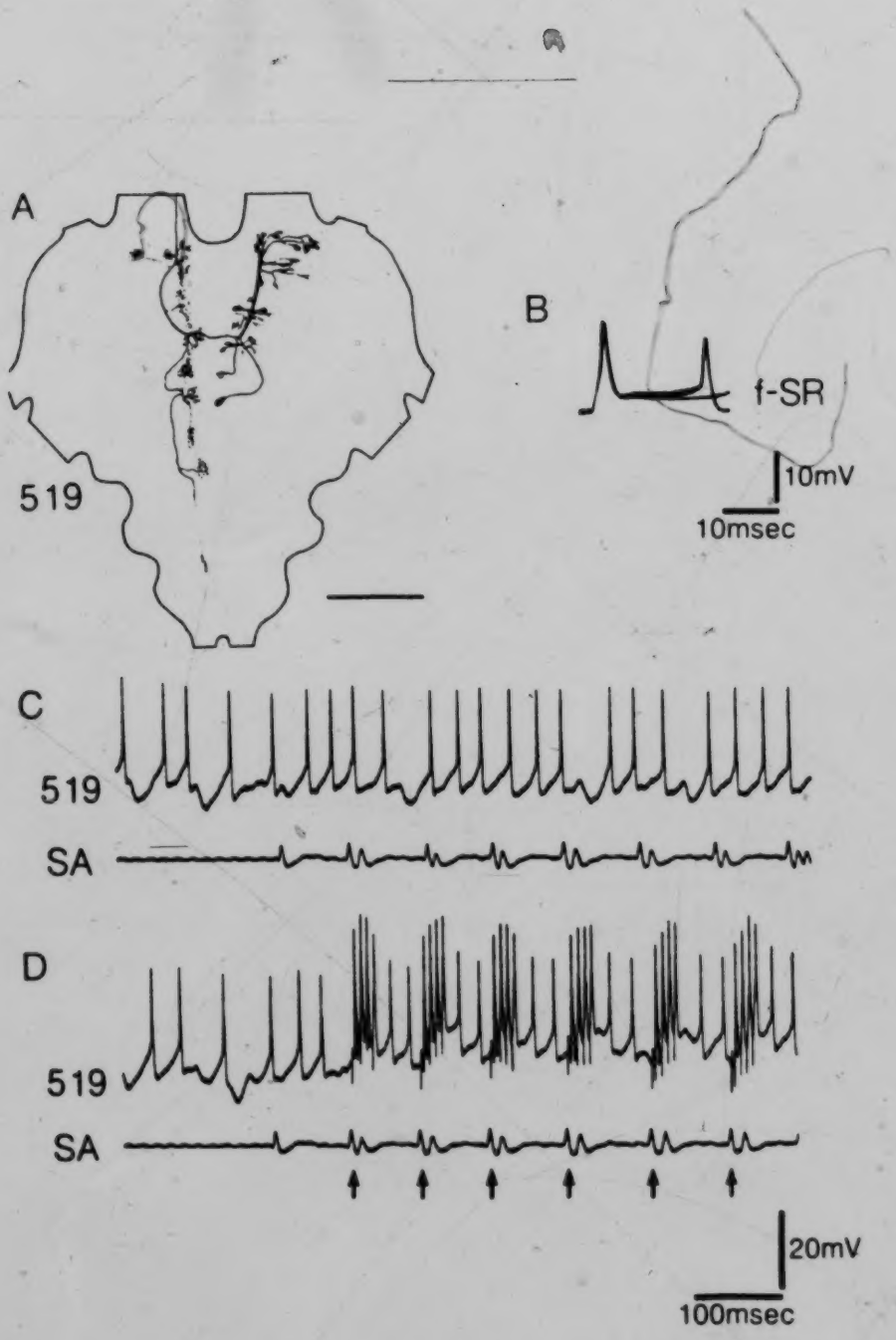
D. DISCUSSION.

Effects of the SRs on Flight Motoneurons.

In *Schistocerca gregaria*, Burrows (1975) found that the SRs evoked short-latency EPSPs in depressor motoneurons and slightly longer latency IPSPs in elevator motoneurons. On the basis of these results, he suggested that the SRs form part of a negative feedback loop by which the amplitude of wing movements could be regulated. Discharge of elevator motoneurons would raise the wing, which would then excite the SRs. The SRs would in turn feedback to inhibit the elevator motoneuron and excite the depressor motoneurons, thus promoting the onset of the downstroke.

The SRs also form short-latency connections to depressor motoneurons in *Locusta* and thus, at least a part of the negative feedback loop could operate in this species.

Figure 48. SR input to a neuron not driven by the central oscillator (519). A : Structure of 519 in the metathoracic ganglion. B : The ipsilateral f-SR evokes a large (6mV) EPSP with superimposed spikes in 519. C : Directing wind to the head evokes flight activity shown by the subalar muscle recording (SA) but there is no response in 519. D : Triggering f-SR stimulation to occur on every oscillator cycle induces rhythmic activity in 519. The spontaneously occurring spikes in 519 are still evident between depolarizations.


A
519
B
f-SR
10mV
10msec
C
519
SA
D
519
SA
20mV
100msec

However, with the exception of the forewing first basalar, these EPSPs were of small amplitude (1mV or less) compared to the rhythmic oscillations seen in these cells during the deafferented rhythm (10 - 15mV) and one may question whether the direct connections by themselves could influence these neurons. However, the SRs not only directly connect to the depressors but they also reduce the repolarization of the depressor motoneuron after it has discharged. Thus the baseline level of depolarization in the depressor motoneurons is increased. One may speculate that the direct connections from the SRs to the depressor motoneurons would now be more effective in influencing the timing of the depressor firing. Hence, the infuence of the SRs on the time of onset of the depressor burst comes from two different pathways. There is the short-latency pathway that is involved in the negative feedback loop of Burrows (1975). The SRs of a particular wing fire before the depressor motoneurons of that wing. The SRs, by their direct connections to the depressor motoneurons (and possibly also to excitatory premotor interneurons) will promote the onset of the depressor burst. Thus, for example, if the amplitude of a particular wingstroke is high, there is an increased SR discharge frequency (Wendler 1983, Möhl 1985b) and an increased excitation to the depressor motoneurons, leading to earlier firing of the depressors. Möhl (1985b) has analyzed the relationship between the time of SR discharge and the time of firing of the ipsilateral depressor motoneurons in intact tethered locusts. An earlier onset of the SR discharge was correlated with an earlier firing of the depressors.

The second part of the negative feedback loop of Burrows (1975) was that the SRs would turn off the elevator discharge. However, I found that the SRs did not evoke IPSPs in elevator motoneurons. The SRs did inhibit the following elevator discharge if the stimulus occurred close to the time of the depressor burst. Also, both the ipsilateral and contralateral SRs had this action. The mechanism by which this effect is produced is unknown. It may be via an excitation of inhibitory premotor interneurons but could also be an inhibition of excitatory premotor interneurons. The significant finding is that the SR

discharge in one elevator-depressor sequence influences the characteristics of the next elevator-depressor sequence by its long-latency influence on both elevator and depressor motoneurons. As both the ipsilateral and contralateral forewing SRs had these effects on motoneurons, the long-latency pathway appears to involve an integration of SR information from more than one wing. An important consideration is the very short cycle times involved in locust flight (wingbeat periods of 40 - 50 msec). Inherent delays within the system occupy a significant proportion of the flight cycle. There will be a considerable lag between the time of a motoneuron's command to a muscle and the time at which information concerning that movement will reach the central nervous system. For example, the SRs signal information concerning the wing elevation, yet they do not discharge until approximately half a cycle following the elevator motoneuron discharge (Möhl 1985b). This greatly restricts the ability of the nervous system to influence the ongoing phase of a movement. One possibility is that the the long-latency pathways are responsible for coarse adjustment of the next wingstroke. Short-latency pathways may be responsible for finer adjustments. For example, the SRs reduce the following elevator depolarization. The tegula, which fires toward the bottom of the downstroke just preceding the elevator discharge (Neumann 1985), may determine the actual firing time of the elevator motoneurons by its short-latency excitation of these neurons (Kien and Altman 1979, Neumann 1985).

Effects of the SRs on Interneurons

The motoneuron data and the results presented in the preceding chapter established three effects of the SRs on the central flight pattern: an influence on the timing of the central rhythm generator, a decreased level of repolarization of depressor motoneurons and a reduced depolarization of elevator motoneurons. Unfortunately, our knowledge of the circuitry underlying the flight pattern generator is far from complete and the cellular mechanisms by which these effects are produced remain unknown. In a first step to elucidating these mechanisms, I have identified many interneurons which receive short,

constant-latency PSPs from the wing SRs. The latencies of the EPSPs were in the same range as those found to depressor motoneurons in *Locusta* and in *Schistocerca* (Burrows 1975). Recently, Peters et al (1985) have directly shown with electron microscopy that at least one of these connections is truly monosynaptic. Hence, it seems likely the EPSPs found in this study were monosynaptic.

Where tested, all interneurons received convergent SR input from at least two wings. This is significant in considering how proprioceptive input is processed by the central nervous system. Each SR is capable of providing very precise information concerning the wing movement (Gettrup 1963, Wendler 1983, Möhl 1985b), yet this information does not appear to be kept separate by the central nervous system. Rather, the interneurons considered here all received SR input concerning more than one wing and this information would arrive at the cell at essentially the same time. Presumably, the input from the SRs of different wings summate and produce a signal related to the movement of at least two wings. There was no consistent pattern of convergence. Most of the interneurons received input from the forewing and hindwing SRs. However, convergence of segmental SR pairs were also observed.

Cell 308 was interesting in this regard. It appears to compare the SR activity on the two sides of the body and may be involved in bilateral co-ordination. For example, its different responses to the ipsilateral f-SR and the contralateral f-SR suggests that it may be sensitive to whether one forewing has commenced the downstroke earlier or later than the other forewing. Remember that the 308s are represented bilaterally. The ipsilateral f-SR to each 308 produces a delayed excitation in each cell (Fig.45c). On the other hand the contralateral f-SR inhibits this delayed excitation (see bilateral response in Fig.45c). Assume the left forewing moves downward before the right forewing. Downward movement of the wing ends the SR discharge. In this case, then, the left f-SR has ceased firing but the right f-SR has not. Now consider the response in each of the 308s. The left 308 will still be receiving the inhibition from the right (i.e. contralateral) f-SR. This will

shunt the excitation produced by the left (i.e. ipsilateral) f-SR. Hence the left 308 will stay inhibited. The situation is opposite for the right 308. The right f-SR (ipsilateral to this 308) is producing a delayed excitation. Because the left (contralateral) f-SR has ceased firing the inhibitory shunt is also removed, further aiding in the excitation of this 308. The net result would be that the left 308 (ipsilateral to the leading forewing) will stay inhibited while the right 308 (ipsilateral to the lagging forewing) will be excited. By this dual response of ipsilateral excitation with contralateral inhibition, differences in time between the end of the two f-SR bursts could lead to differences in the time between repolarization of the two 308s which could then direct appropriate adjustments in the motor output. Obviously, a future experiment would be to examine whether current injection into this cell can change the relative timing of the motor output on the two sides of the body.

The convergence of proprioceptive information from different wings to the same interneurons is consistent with what is known of the cellular organization of the central rhythm generator (Robertson and Pearson 1983). By far the majority of flight interneurons so far discovered are interganglionic and premotor interneurons often connect with both forewing and hindwing motoneurons. Robertson and Pearson (1983, 1985) have presented evidence suggesting that the movement of all four wings is controlled by the same oscillator network. The proprioceptive convergence found here in turn suggests that adjustments of the motor output are made in relation to information coming from several wings rather than each wing being controlled separately. Supporting evidence for this conclusion has come from other studies on locust flight proprioceptors. For example, Gettrup (1966) found that the hindwing campaniform sensilla were necessary for the control of the forewing angle of attack, by which the locust regulates lift. Electrical stimulation of the forewing campaniform sensilla evokes contractions of fore- and hind-wing depressor muscles (Wendler 1978, Horsmann and Wendler 1985). Electrical stimulation of the hindwing tegula also has been found to influence both forewing and hindwing motoneurons (Kien and Altman 1979).

The pattern of SR connections to interneurons described here may be relevant to the results presented in chapter three. In most cases, individual interneurons received SR input from only one side of the animal. This may partly explain why SR stimulation from one side was a weak entraining stimulus, whereas much stronger effects could be seen with bilateral stimulation. The common convergence of the forewing and hindwing SRs to the same interneurons suggest that both segmental SR pairs entrain the rhythm through the same set of interneurons. The observation was also made that entrainment with hindwing SR stimulation tended to be less stable than from the forewing SRs. Correlated with this was the fact that they do show some differences in their connections to central interneurons (e.g. cells 313 and 308). Whether these neurons are involved in resetting the rhythm is unknown.

Most of these connections were to neurons depolarized in phase with depressor motoneurons. The SRs are active on every flight cycle and their activity occurs close to the occurrence of depressor activity (Möhl 1979, 1985b). Therefore these cells will already be depolarized by the central oscillator when the SR input arrives at the neuron. How much the SR input can change the depolarization depends on the neuron in question. In some cases, SR input arriving coincident with the centrally derived depolarization did not seem to have any effect on the depressor's discharge pattern (Fig.37). The function of these connections may be to ensure that the spike rate of the cell is of sufficient frequency or to cause fine adjustments in the onset of the burst. However, other depressors were more strongly influenced by the SRs, so that the centrally derived depolarization was prolonged or increased (Figs.38,39,41). The most surprising result of this study has been that elevator and non-rhythmic interneurons receive very powerful inputs from the wing SRs. As the SRs are active on every cycle during flight, these cells will be strongly depolarized in phase with the SRs, that is, close to the time of depressor activity. This can result in a double burst pattern in at least one interneuron (313, Fig.43) or the occurrence of spontaneously occurring spikes between bursts (519, Fig.48). It seems unlikely that this

situation would occur in the intact animal and I speculate that other proprioceptors may act to inhibit these cells between the SR-evoked bursts. For example, this could be produced by either the tegula or the campaniform sensilla which discharge during the downstroke (Gettrup 1966, Neumann 1985). This seems to be the case for 308. When SR stimulation was presented on every cycle, its rhythmic depolarizations were abolished. However, it receives a strong excitatory input from unidentified afferents in meso.nlc, which carries the afferent axons from the tegula and campaniform sensilla (Altman et al 1978)

Cell 519 was the only neuron which was found to receive SR input but was not active during the central flight rhythm. Because the SRs are active on every cycle, this cell is presumably involved in flight control in the intact animal. Since the SRs are only one group of proprioceptors involved in flight, it may be that there is a population of flight interneurons which do not receive any central oscillator input but which are rhythmically excited by proprioceptors in the intact animal. Thus, the neural circuits in the thoracic ganglia which underly control of locust flight very likely consist of more neurons than are revealed by looking at only the responses of neurons active during the deafferented rhythm.

The striking finding has been that proprioceptive input can cause significant alterations in the patterns of activity of particular interneurons. Mimicking input from the SRs can produce prolonged bursts, increased discharge frequency, abolition of activity and changes in the phase of firing. Certainly, the SRs do not seem to be merely making small corrections of a centrally generated pattern. Rather, the activity of some interneurons is quite re-organized. It would appear that a number of interneurons likely function in a different manner to that suggested by their activity in deafferented preparations.

V. THE ROLE OF THE SRs IN THE MAINTENANCE OF FLIGHT.

A. INTRODUCTION.

Wilson and colleagues (Wilson and Gettrup 1963, Wilson and Wyman 1965) suggested that the wing SRs increased the wingbeat frequency by increasing the general excitability of the central nervous system. The results presented in the previous chapters suggested that the SRs increased the flight frequency by a phase-dependent action on the central flight rhythm generator. However, two observations may possibly reflect actions of the SRs on other sites of the central nervous system which influence the flight rhythm. Firstly, triggered SR stimulation not only increased the frequency of the rhythm but also prolonged the duration of flight sequences. The second observation was that presentation of SR stimulus trains at high rates could elicit a slow, phase-independent increase in flight frequency as was reported in earlier studies (Wilson and Gettrup 1963, Wilson and Wyman 1965; chapter three). Recently, Möhl (1985c) has performed experiments which he concludes support the notion of a phase-independent pathway by which the SRs can influence the flight frequency. In intact locusts, he stimulated one forewing SR afferent nerve (meso.n1d_2) during tethered flight. He found that stimulation of this nerve every cycle (triggered from the hindwing depressor muscle spike) could cause changes in the wingbeat period independently of the time in the cycle at which the stimulus was given. He reported a great deal of variability between animals but the most common response was a rapid decrease in period of approximately 1 millisecond which was maintained for the duration of the stimulation. Möhl's (1985c) experiments together with the observations of Wilson *et al* raise the question of whether other pathways exist by which the SRs could influence the flight frequency apart from their direct effects on the flight rhythm generator.

In this chapter, I describe a set of interneurons which receive stretch receptor input and whose properties suggest a separate pathway by which the SRs could influence the flight frequency. These neurons have been called 404s. The structure and physiology of these neurons

are described and evidence is presented for their being involved in the initiation and maintenance of the flight rhythm. They fired tonically throughout a flight sequence. Depolarizing a 404 could initiate flight activity and hyperpolarizing a 404 could sometimes prevent the initiation of flight. Hyperpolarizing a 404 during flight either slowed or stopped the flight rhythm. The response of these neurons to SR stimulation during rhythmic flight activity was examined. It was found that the prolonged duration of flight sequences elicited by SR stimulation was accompanied by a prolonged discharge in this group of interneurons. Possible mechanisms by which SR stimulation maintains the activity in these neurons were investigated. It is concluded that neither a direct excitation nor positive feedback from the central oscillator could account for the prolonged activity in the 404s. The actual mechanisms remain unknown.

B. MATERIALS AND METHODS.

The preparation used in these experiments is the same as described by Robertson and Pearson (1982) and used in chapter four for intracellular recording from interneurons during the deafferented flight rhythm. The forewing SRs were stimulated bilaterally at their meso.n1d$_2$ or pro.n6 projections. In some cases, the stucture of the 404s was examined in histological sections. Preparations containing well-stained neurons were embedded in low-viscosity resin (Spurrs), sectioned (10 - 15μm thick sections) and counterstained with 1% methylene blue and azure blue. Fiber tracts were named according to the nomenclature of Tyrer and Gregory (1982).

C. RESULTS.

Properties of the 404s

The interneurons of interest in this chapter formed a small group in the anterior mesothoracic ganglion. They had several anatomical and physiological properties in common. Neurons of very similar structure have been described by Watson and Burrows (1983) in the

closely related locust species, *Schistocerca gregaria*. Figure 49 illustrates the major structural features shared by the neurons of this group in *Locusta*. The cell body was located at the antero-lateral surface of the mesothoracic ganglion. From the soma, a neurite ran posteriorly and dorsally to the dorsal part of the ganglion where the major arborization was located. Prominent was a neurite which ran transversely, just above the dorsal medial and dorsal intermediate tracts. Laterally, this process formed a loop around the lateral dorsal tract, giving rise to the axon, which projected anteriorly in the ipsilateral mesothoracic-prothoracic connective. Because the axon was ipsilateral to the soma and directed anteriorly these cells were labelled 404s according to the numbering scheme of Robertson and Pearson (1983). Intracellular recordings were made from the dorsal neuropil processes of the 404s. Data were obtained from 40 recordings of these neurons.

In addition to these shared structural features, the 404s also shared the following physiological properties (Fig.50).

1. They discharged tonically during a flight sequence elicited by a windstream to the head (Fig.50a). Occasionally the 404 spike rate was weakly modulated with the flight rhythm (e.g. Fig.50a). However, this modulation was very slight and was apparent in only a few cases.
2. They were transiently excited by sudden changes in the ambient illumination. The response to decreased illumination was stronger than to increased illumination (cf Figs.50b and 50c).
3. They were weakly depolarized by hissing sounds.

The first indication that there was more than one type of 404 in each hemiganglion was a variability in the dorsal neuropil branching of neurons recorded in different animals. This was also noted by Watson and Burrows (1983). However, it was possible to categorize the different encountered structures into one of four morphological types. These different types are shown in Figure 51. While all shared the same basic anatomical plan described above (Fig.49), there were differences in their secondary branching pattern. For example, the interneuron shown in Figure 51a (404A) had a thick medial process directed posteriorly but no process arose from the lateral loop, while the 404 of Figure 51b (404B) had a thinner corresponding medial branch and

Figure 49. Drawings showing the main structural features of the 404 neurons. A: Dorsal view of a 404 neuron in the mesothoracic ganglion (anterior top). The axon projects anteriorly in the connective ipsilateral to the soma and the main neurite has a characteristic looped appearance. B: Posterior view (dorsal top) showing the path of the main neurite relative to major nerve tracts. The neurite runs dorsal to the dorsal intermediate tract (DIT) and the dorsal medial tract (DMT) and loops around the lateral dorsal tract (LDT). C: Trajectory of the main neurite of a 404 neuron as seen from the side.

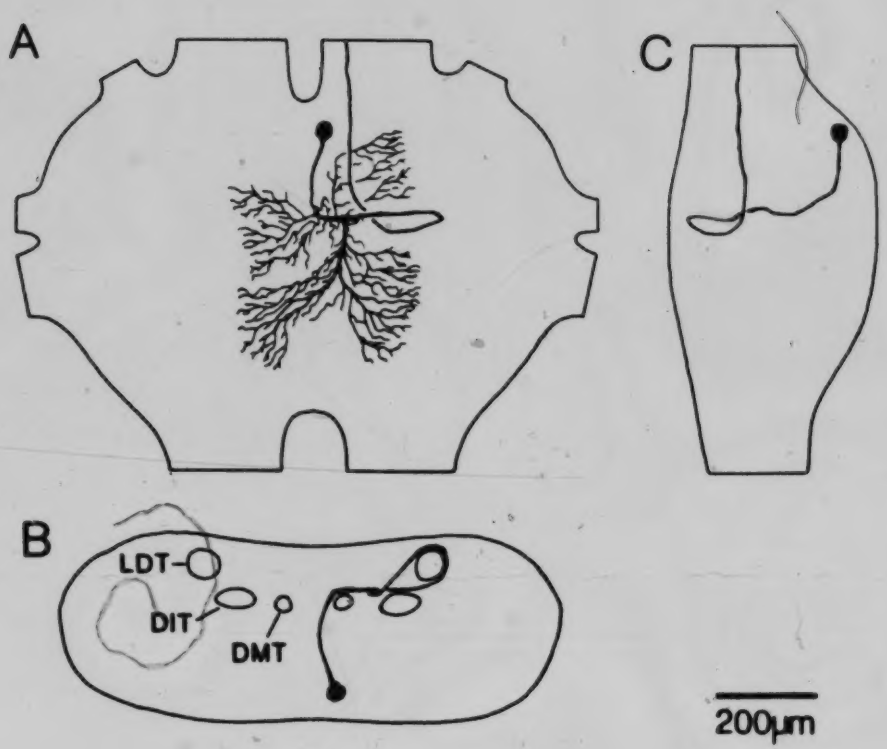

A
C
B
LDT
DIT
DMT
200µm

Figure 50. Common physiology of the 404 neurons. A : Response of a 404 to a constant windstream directed to the head. Upper trace : intracellular recording from a 404. Lower trace : depressor EMG (hindwing dorsal longitudinal muscle). B : Response in 404 to turning off the lights (different animal from that recorded in A) C : Response in the same 404 as in B but to turning on the lights. Note that no depressor activity was evoked, corresponding with only weak depolarization seen in the 404. Vertical calibration : A : 20mV ; B,C : 40 mV Horizontal calibration : A : 1sec ; B : 0.5sec.

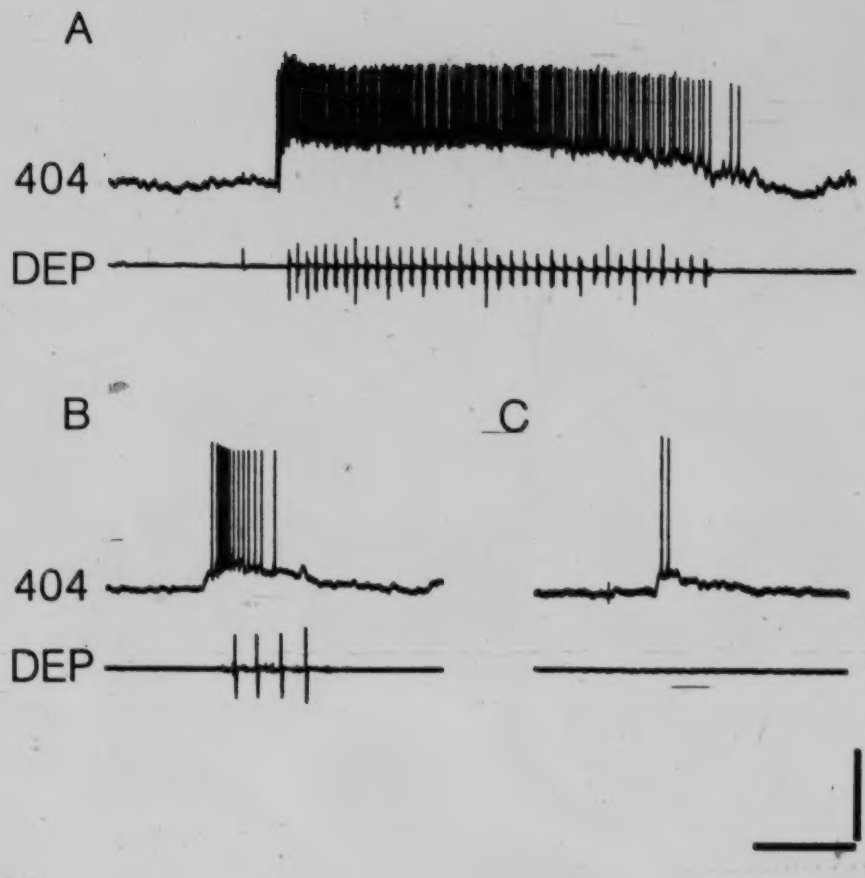
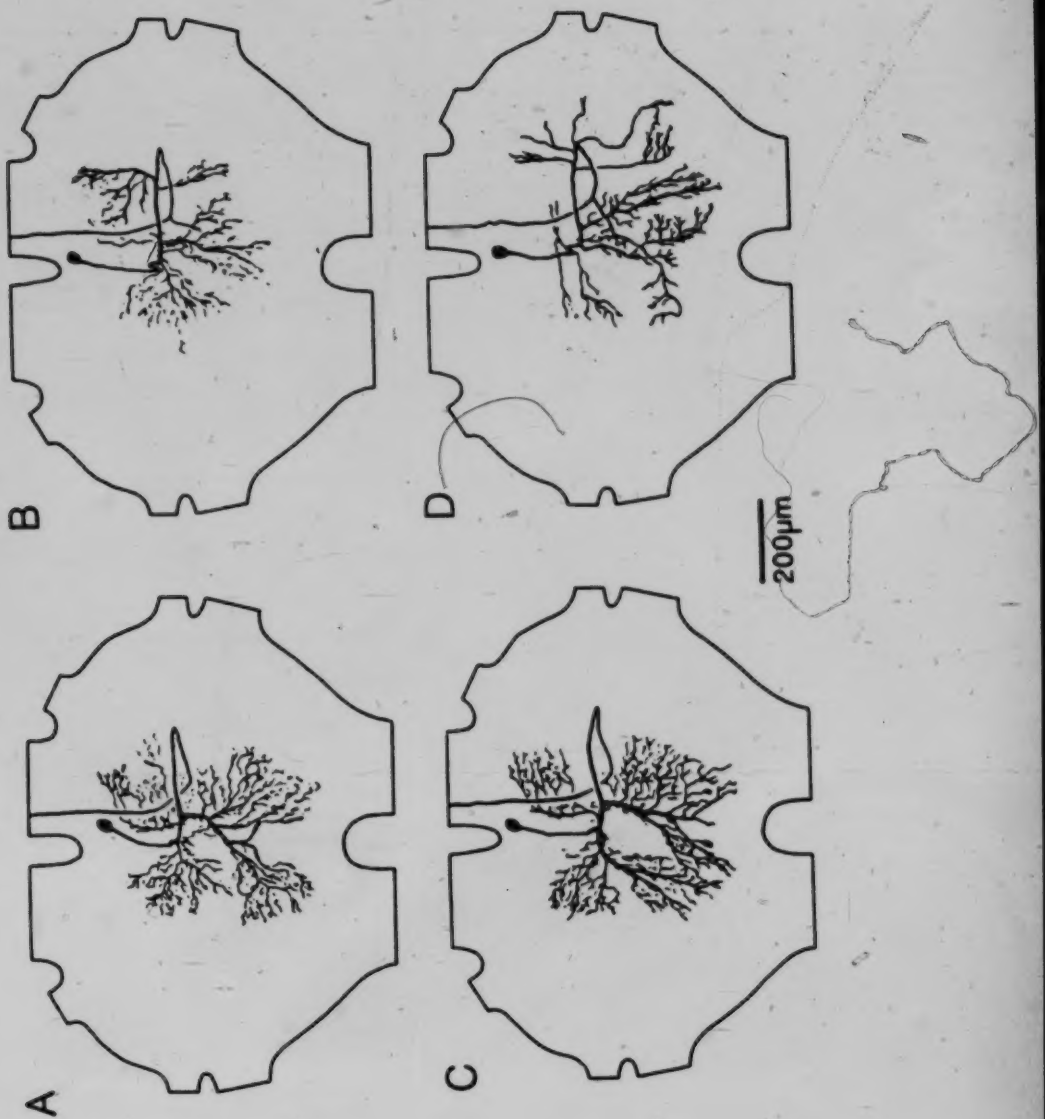

A
404
DEP
B
C
404
DEP

Figure 51. The different morphological types of 404. Four structural types of 404 neuron have been identified. All possess an anterior cell body at the ventral surface of the mesothoracic ganglion as well as a looping lateral neurite with the axon ascending in the ipsilateral mesothoracic - prothoracic connective. Differences were found in their secondary branching. The types shown in A and B were proven to be different neurons. It was not established that types C and D were different neurons or whether they were variations of a single neuron type.


B
D
200µm
A
C

also had an anteriorly directed branch arising laterally from the looping process. The types in Figures 51c (404C) and 51d (404D) also showed differences from these two types. Furthermore, it was possible to correlate these different structural classes with different responses to sensory stimuli. For example, 404A was weakly excited by touching the abdomen while 404B was inhibited by that stimulus. The different 404 structures could be distinguished by examining their responses to blowing on the cerci and to touching the abdomen. The response of each type is summarized in Table III.

The two most commonly encountered 404 types were 404A and 404B. That these were indeed different neurons was proven by filling both neurons in the same hemiganglion in four different preparations. The situation was not as clear for 404C and 404D. The neuron 404C had a very similar structure to that of 404A, except that it possessed a prominent branch, which projected postero-laterally on the side contralateral to the loop. Correlated with this was an apparently stronger response to blowing wind on the cerci. However, these comparisons were between animals and it is not possible to say with certainty that 404A is distinct from 404C. Neuron 404D possessed a quite distinct structure (Fig.51d) and was also the only 404 to be inhibited by blowing on the cerci. However, this neuron was stained in only one animal and hence it is also uncertain whether it represents a distinct 404 type. Thus, it is only known with certainty that there are two 404 types per hemiganglion, but it is very possible that others exist.

One piece of evidence suggested the maximum number of 404s may be 5 per hemiganglion. This was based on histological examination of sagittal sections through the looping lateral neurite (Fig.52). These sections revealed that the main transverse neurite ran in a small tract containing five profiles of similar diameter. One of these profiles was the labelled 404 neuron. In cases where two 404s were stained, both were contained within this tract. Not all these profiles necessarily belonged to 404 neurons. However, if all 404s project in this tract, then the maximum number of 404s would be five per hemiganglion.

TABLE III - Correlation of different morphological types of 404 with different responses to sensory stimulation. In the table, the 404s are distinguished by an alphabetical character which refers to the corresponding structure in Figure 51. All 404s were excited by blowing wind on the head. 404B could be distinguished from the other types by its strong hyperpolarizing response to touching the abdomen. 404D was the only type to be inhibited by blowing wind on the cerci. Neurons 404A and 404C had very similar responses to all three stimuli but 404C had an apparently stronger response to blowing on the cerci. However, this is a subjective impression and no attempt at a quantitative comparison has been made.

++++ : very strongly excited

+++ : strongly excited

++ : moderately excited

+ : weakly excited

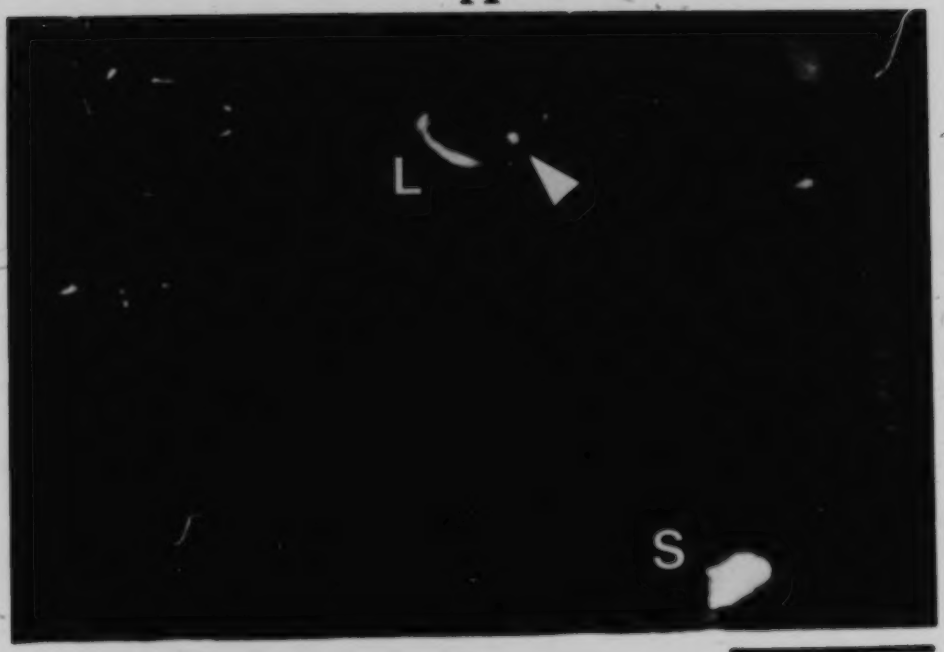
- : inhibited

TABLE III

	404A	404B	404C	404D
WIND ON HEAD	++++	++++	++++	++++
ABDOMEN	+	-	+	+
CERCI	++	++	+++	-

Figure 52. Micrographs of saggital sections through a mesothoracic ganglion containing a 404 stained with Lucifer yellow. Sections were taken at a level ca. 80μm lateral to the midline. Dorsal is up and anterior to the right. A : Distribution of fluorescently labelled 404 processes. Indicated are the cell body at the antero-ventral margin of the ganglion (S), the transverse neurite (arrow) and part of the process which loops around the lateral dorsal tract (L) before projecting in the mesothoracic-metathoracic connective. B : The same section as in A at higher magnification and increased background illumination. This shows that the stained transverse neurite (arrow) runs in a small tract above the dorsal medial tract. Note the presence of four other profiles of similar diameter within this tract. Calibrations: A: 100μm, B: 50μm.

A



B

Role of 404s in Flight Initiation and Maintenance.

Commonly, on first penetrating a 404, flight activity would commence. This was correlated with the initial injury discharge of the 404 upon penetration by the microelectrode. The rhythmic firing of the depressor muscles could be abolished by hyperpolarizing the neuron during this period. Conversely, releasing the hyperpolarizing current, initiated a sequence of depressor EMG bursts (Fig.53). Once the penetration had stabilized so that there was little or no spontaneous 404 activity, flight activity could be initiated by depolarizing the 404 (Fig.54). The currents required were usually quite high (7 - 10nA). The associated 404 spike frequency was in the range of 80 - 120 Hz before flight was initiated, compared with 60 - 80 Hz as the initial 404 response to flight initiated by wind to the head. The higher levels of 404 activity required to initiate flight activity could be most simply explained as being due to the fact that wind stimulation excited all 404s, while only one 404 was being depolarized with intracellular current injection. One possibility was that depolarizing 404s simply caused a tonic firing of dorsal longitudinal motoneurons. To show this was not the case, intracellular recordings were made from a hindwing depressor motoneuron (first basalar) while depolarizing a 404 neuron (Fig.55). Clear rhythmic membrane potential oscillations were induced by the stimulus, with a similar shape to those seen when flight was elicited by a windstream to the head (Fig.55a). The only difference in this case was that the depressor only fired one spike per cycle, rather than two. Thus, the 404s were sufficient to initiate flight activity.

However, it was not possible to unequivocally demonstrate that they were necessary. That is, hyperpolarizing individual 404 neurons often could not prevent the initiation of flight activity. This was particularly the case with a windstream directed to the head, although if wind puffs were light and of short duration, hyperpolarizing a 404 could sometimes prevent flight activity being initiated. The results were more consistent for weak flight-initiating stimuli such as turning off the lights (Fig.56), although not in every case. Thus, individual 404s were not always necessary for flight initiation. Again, it could be argued that only one 404 was being influenced by the current injection and that the other 404s were responsible for flight initiation

Figure 53. Initiation of flight associated with high frequency discharge of 404. Upper trace : intracellular recording from 404, AC coupled. Lower trace : depressor EMG (hindwing DLM). Recording from a 404 shortly after penetrating the neuron. In this case, there was a prolonged period of injury discharge. At the beginning of the trace shown, the 404 was hyperpolarized (about 5 nA) to prevent action potentials in the cell. There was no activity in the DLMs. Where indicated ("hyp off"), the hyperpolarization was released. Shortly later, rhythmic bursts were recorded in the DLM. The DLM activity ceased shortly after restoring the hyperpolarizing current ("hyp on"). The breaks in the intracellular record were due to saturation of the amplifier during the switching off and on of the current.

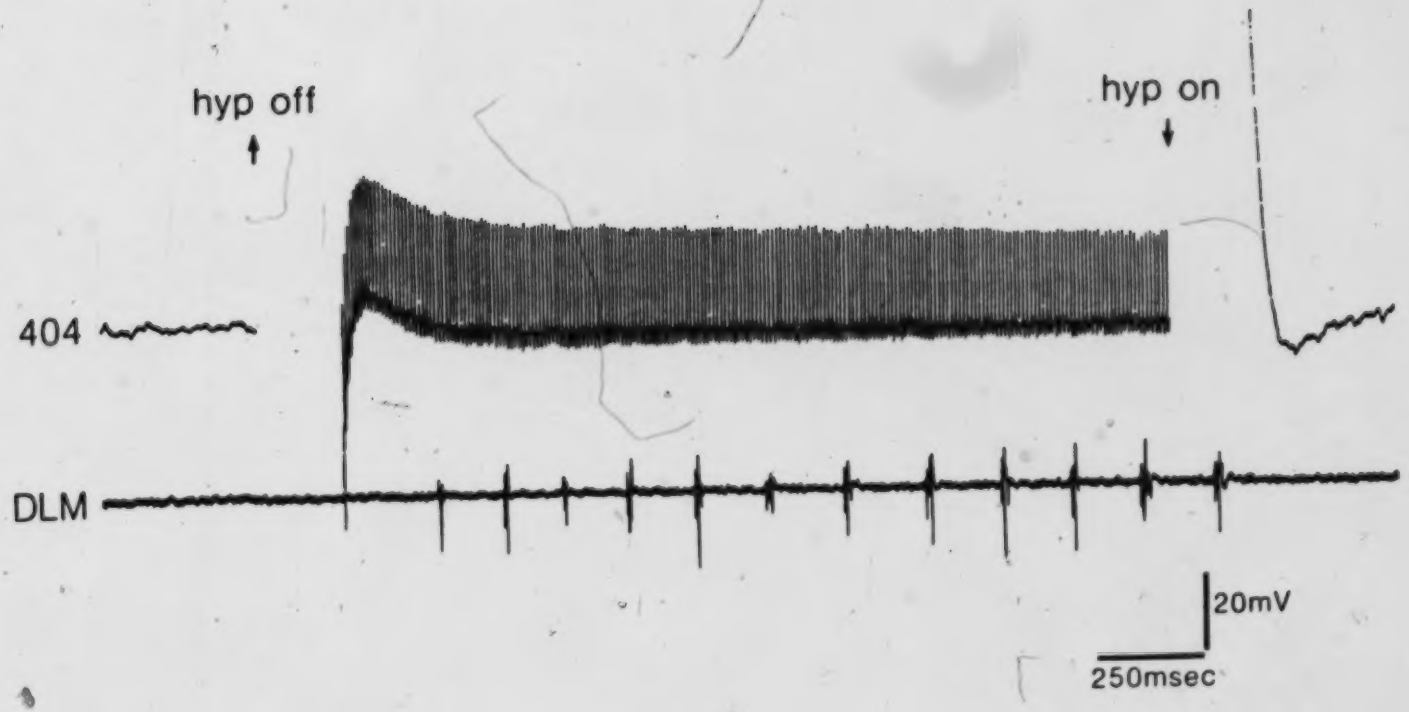

hyp off
hyp on
404
DLM
20mV
250msec

Figure 54. Initiation of flight activity by depolarizing a 404 neuron. Middle trace : intracellular recording from a 404 (AC coupled) while injecting an increasing amount of depolarizing current. The associated instantaneous 404 spike frequency is shown in the upper trace. In this case, rhythmic firing of the depressors (DLM, lower trace) commenced when the 404 frequency reached about 80 Hz.

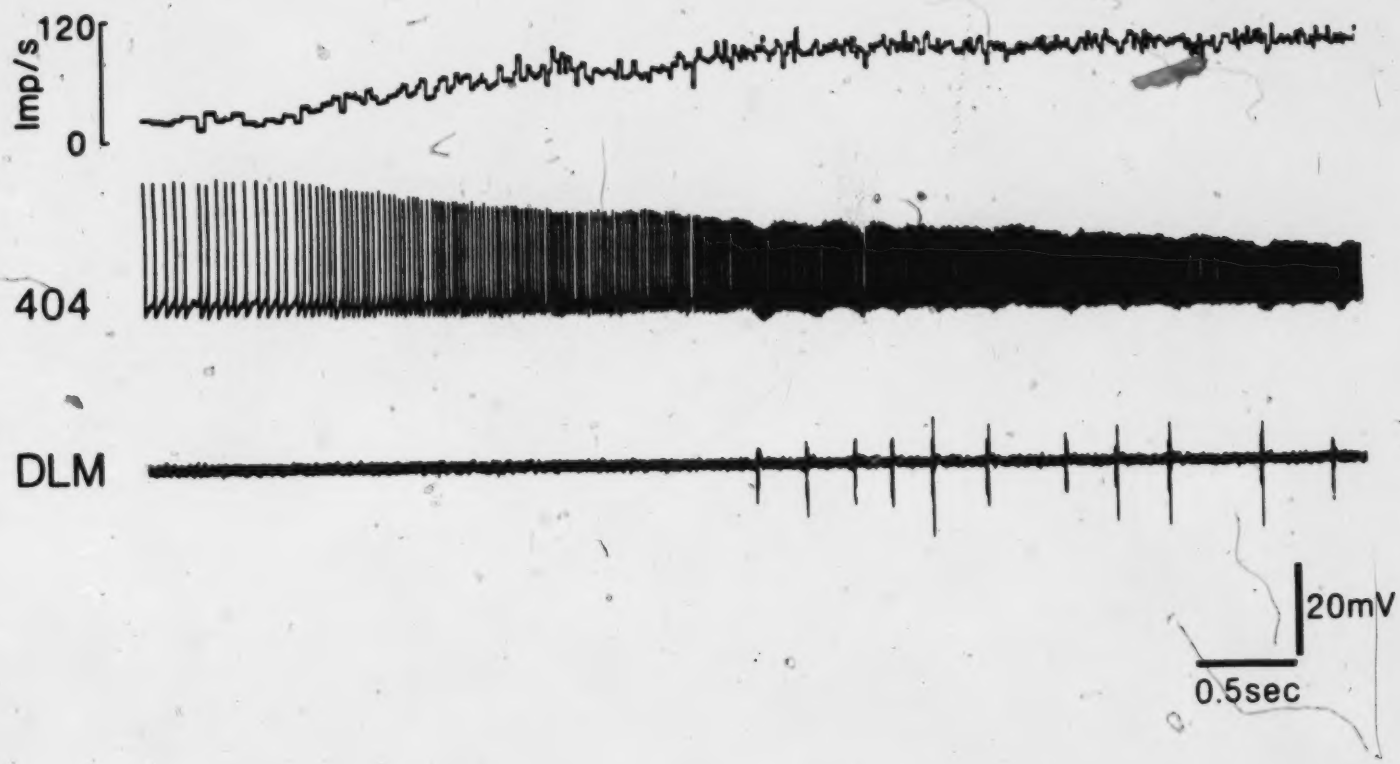

Imp/s
120
0
404
DLM
20mV
0.5sec

Figure 55. Rhythmic membrane potential oscillations in a depressor motoneuron elicited by depolarizing a 404 neuron. Simultaneous intracellular recordings from a 404 (upper trace) and a metathoracic depressor motoneuron (1st Basalar; middle trace) as well as the depressor EMG (DLM) during flight activity elicited by directing a windstream to the head. B : As in A, but instead of directing wind to the head, the 404 neuron was depolarized. Note that the membrane potential oscillations in the depressor motoneuron were of very similar shape to that seen with wind directed to the head, though only one spike was elicited per cycle, rather than 2 spikes as seen in A. The 404 recording in B was AC coupled.

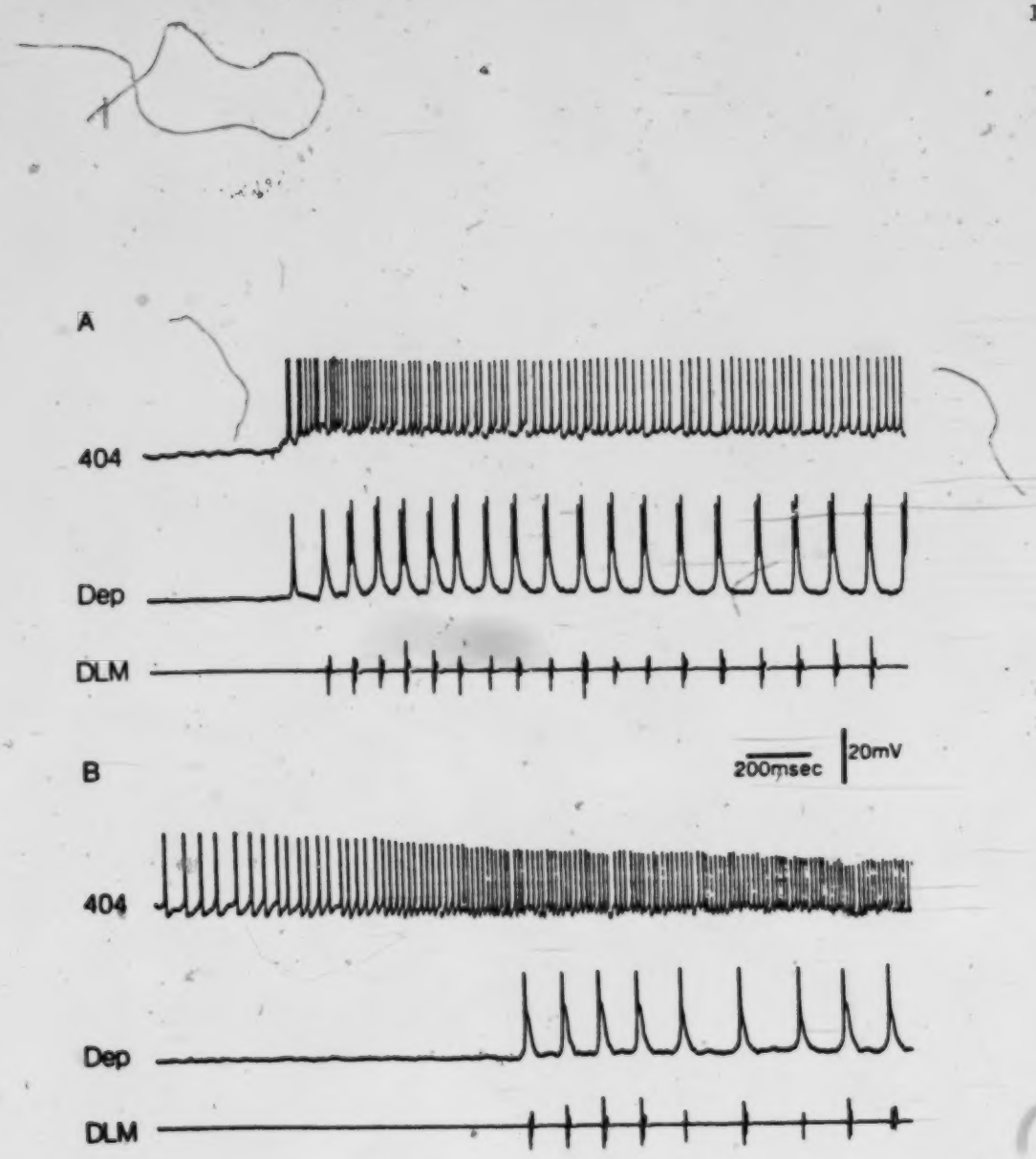
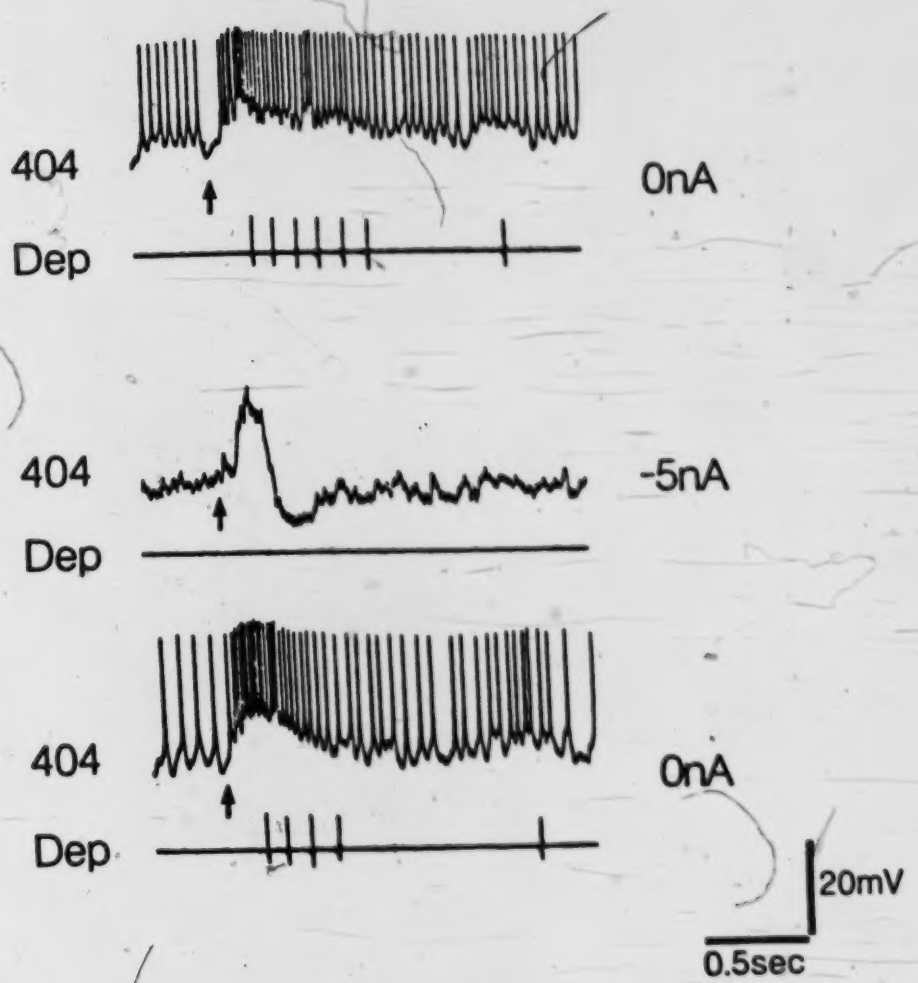

A
404
Dep
DLM
200msec
20mV
B
404
Dep
DLM

Figure 56. Inhibition of flight initiation by hyperpolarizing a 404 neuron. Three traces from the same animal. The upper trace in each case is an intracellular recording from a 404. Lower trace is the EMG recording from the hindwing depressor (subalar). Flight activity was initiated by turning the lights off (indicated by the arrow). A : Turning lights off elicits a burst of activity in the 404 and 6 bursts in the subalar. B : The 404 was then hyperpolarized (the 404 trace is AC coupled here) to prevent spiking activity. The stimulus now does not evoke firing in the depressor. C : The hyperpolarizing current was removed. The stimulus again evokes flight activity.


404
Dep
0nA
404
Dep
-5nA
404
Dep
0nA
20mV
0.5sec

under these conditions.

The evidence presented above suggested the 404s are involved in flight initiation. The question of major interest to this thesis was whether they were also involved in the maintenance of rhythmic activity. This appeared to be the case. The 404s discharged throughout a flight sequence. Termination of 404 activity was followed within one or two cycles by termination of rhythmic firing of depressor muscles. Moreover, the discharge rate of the 404s was strongly correlated with the frequency of the flight rhythm. As the flight frequency declined so did the 404 discharge rate. Plotting the flight rhythm frequency as a function of the 404 frequency revealed the two to be linearly related (Fig.57). The correlation in nearly all cases was high ($>.90$, least squares regression). If the 404s were hyperpolarized during a flight sequence then flight activity sometimes stopped but more commonly the frequency of the rhythm was simply decreased (Fig.58). This was best seen if the preparation maintained a fairly constant average frequency.

A final point before returning to consideration of proprioceptive input is whether part of the 404s' activity represented positive feedback from the oscillator neurons. There was no evidence to support this view. Firstly, during a flight sequence, there were usually no rhythmic fluctuations in membrane potential. In the few cases where this was apparent, the modulation was more characteristic of a weak phasic inhibition of a maintained depolarization than of phasic excitation. Secondly, it was possible to initiate flight activity without strongly depolarizing the 404s (by touching the abdomen). Indeed, one of the 404s (404B) was hyperpolarized by this stimulus even though flight activity was initiated (Fig.59). Further evidence that the 404s do not receive positive feedback from the flight oscillator is presented in the next section.

Influence of the SRs on the 404s

One of the striking effects of the SRs on the flight rhythm is that they greatly prolong the duration of flight sequences elicited by a constant windstream to the head. The properties

Figure 57. Relationship between the flight frequency (ordinate) and the discharge rate of a 404 (abscissa). The two are linearly related. As the 404 discharge rate declined, so did the frequency of depressor EMG bursts. In this case, the correlation was .97 (least squares regression).

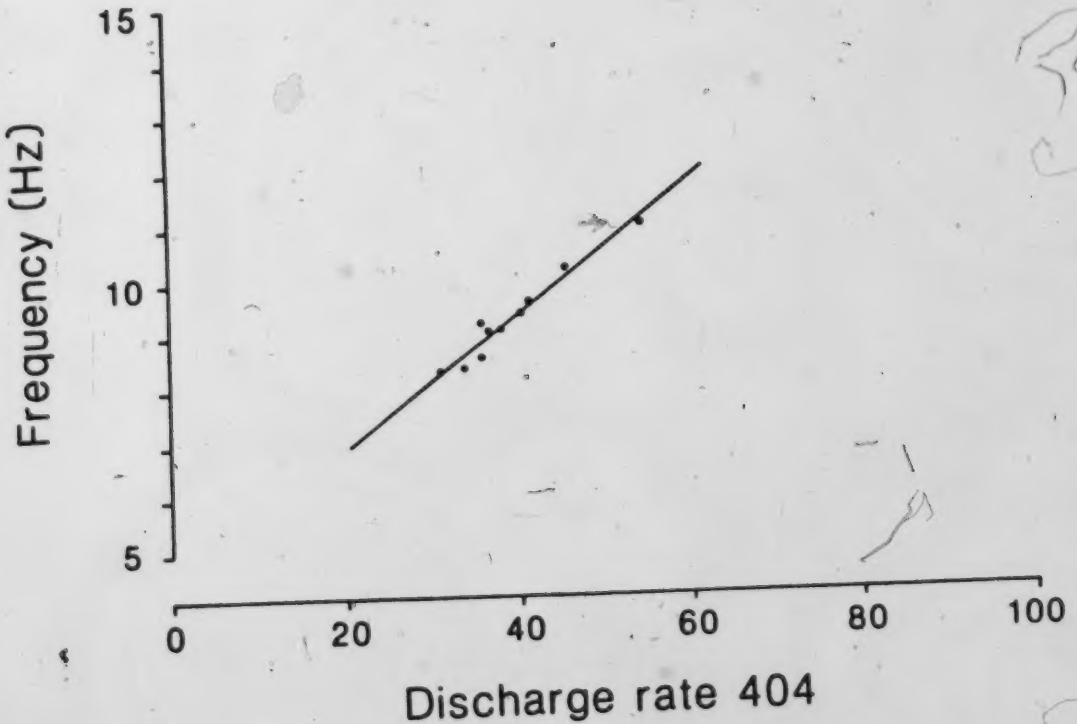
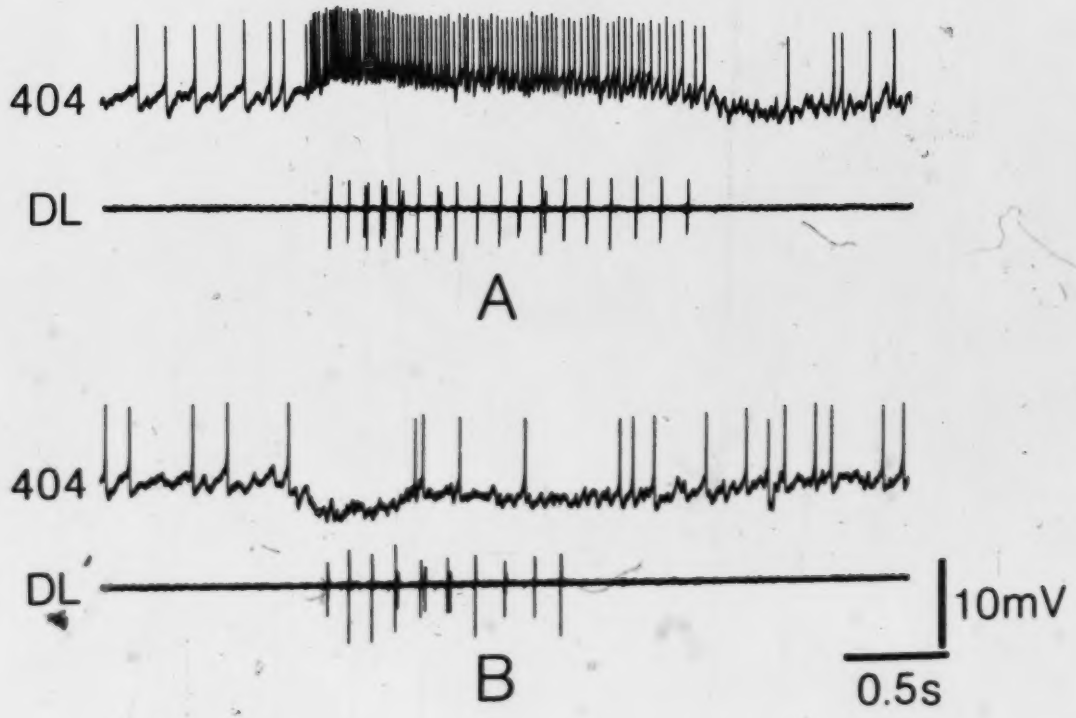

15
10
5
Frequency (Hz)
0
20
40
60
80
100
Discharge rate 404

Figure 58. Effect of hyperpolarizing a 404 on flight frequency. Upper graph : Flight frequency versus time. Each point is the average of 5 cycles. Lower graph : Discharge rate of 404 versus time. Each point is the average discharge rate over 10 spikes in 404. During the flight sequence, elicited by a continuous windstream to the head, the 404 was hyperpolarized for 5 seconds. Onset of the hyperpolarization is indicated by a downward arrow and the end of the hyperpolarizing pulse is indicated by an upward arrow. This pulse was given 4 times during the sequence in this case. Correlated with the onset was a decline in frequency. The flight frequency increased when the hyperpolarization was removed. This was most clearly seen when the flight frequency remained steady.

11
6
flight freq
50
0
404 freq
0
45
Time (sec)

Figure 59. Evidence that the 404s do not receive excitatory input from the central oscillator. A: Response in 404B to wind on the head. B: Response in the same neuron to touching the abdomen. This stimulus evoked flight activity but did not excite the 404.


404
DL
A
404
DL
10mV
0.5s
B

of the 404s suggest that they are an important component of the mechanisms responsible for maintaining flight activity. Therefore the influence of the SRs on these neurons was particularly relevant. Interestingly, then, the ipsilateral fore- and hind-wing SRs evoked constant short-latency EPSPs in some members of the 404 population. These were of small amplitude (1mV or less). They were similar to the EPSPs shown in the previous chapter. There was variation in this connection according to morphological type. Namely, 404A received EPSPs whereas 404B did not. However, 404B was indirectly excited by SR stimulus trains. The degree to which the SRs excited the 404s will be examined later.

The first question of interest was whether the 404s kept discharging during flight sequences prolonged by SR stimulation. Therefore, recordings were made from individual 404 neurons during flight activity with and without accompanying SR stimulation. Figure 60 compares two trials from the same animal. In one trial only a wind stimulus was given. In the other, the SRs were stimulated on every cycle immediately following the hindwing depressor spike in addition to the presentation of a constant windstream to the head. Without SR stimulation the frequency of the rhythm declined over time, which was paralleled by a decline in the 404 frequency. In the trial with SR stimulation occurring on every cycle the flight frequency was increased and the duration of the sequence prolonged. Initially, the 404 frequency decreased but then reached a steady discharge rate which was maintained throughout the flight sequence. This response was observed in both 404A and 404B. Thus, the prolonged duration of flight sequences was correlated with maintained activity in the 404s.

By what mechanisms do the SRs maintain the activity in the 404s? One possibility was that the SRs do this via direct excitation of the 404s. To examine this, SR stimulus trains were presented at a constant rate of 12 - 14 Hz in quiescent animals without accompanying flight activity. This situation mimicked the SR input to the 404s during flight activity accompanied by SR stimulation. Both 404A and 404B were excited by SR stimulation (Fig.61). However, 404A was much more strongly excited than 404B. This would be expected as the SRs evoke short-latency EPSPs in 404A but not in 404B. However, in both 404A and 404B the resultant

Figure 60. The prolonged duration of flight sequences by SR stimulation is correlated with maintained activity in the 404s. Results from two trials in the same animal. In one trial only wind stimulation was given. On the subsequent trial, in addition to wind on the head, the forewing SRs were stimulated bilaterally on every cycle immediately following the hindwing depressor spike. Upper graph: Average flight frequency versus time for the trial without SR stimulation ("wind alone") and the trial with SR stimulation ("wind & stim"). Each point is the average of 5 flight cycles. The SR stimulation increases the frequency and prolongs the duration of the flight sequence. Lower graph: The corresponding 404 discharge rate recorded intracellularly on the same trials (each point is the average of 20 spikes). In both cases the 404 discharge frequency initially declined at a similar rate. Without SR stimulation the 404 frequency continued to drop. However, with SR stimulation on every cycle, the 404 frequency did not decline further but maintained a fairly steady level for the duration of the flight sequence.

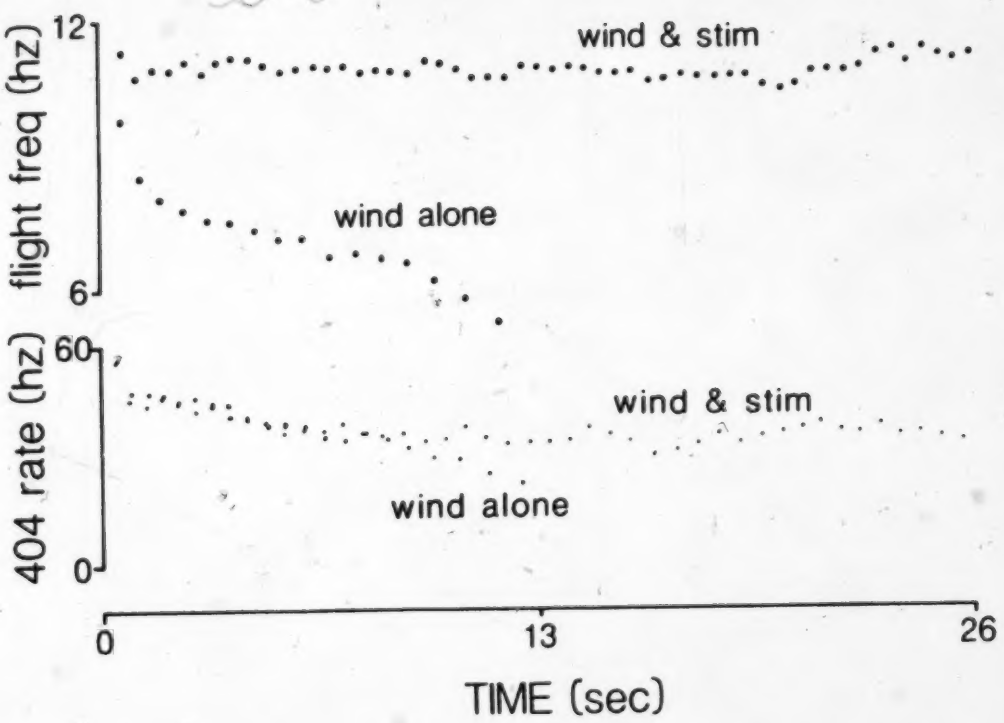
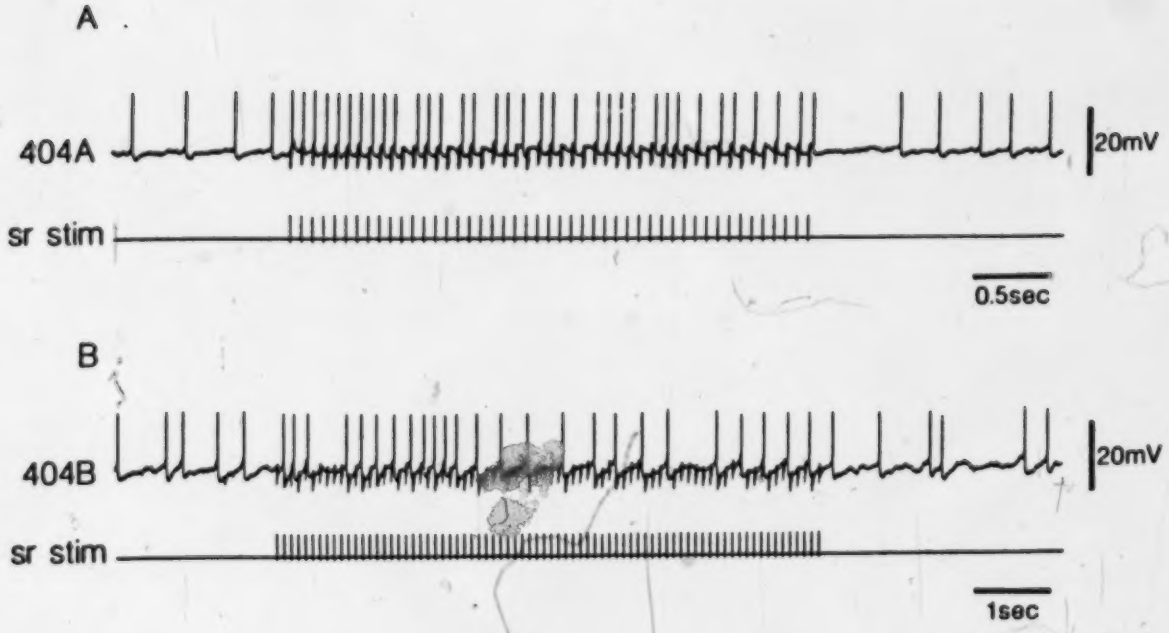

12
6
flight freq (hz)
wind & stim
wind alone
60
0
404 rate (hz)
wind & stim
wind alone
0
13
26
TIME (sec)

Figure 61. Effect of high frequency SR stimulation on 404 activity in quiescent preparations. A: Intracellular recording from a 404A (upper trace) during presentation of SR stimulus trains at approximately 14 Hz. The time of onset of each stimulus train is indicated in the lower trace. The vertical transients on the intracellular record are stimulus artifacts. B: As in A but recorded from a 404B. Stimulus trains were presented at approximately 12 Hz. Note the slower time scale in B. Both 404s were excited by the stimulation but the response in 404A was considerably stronger than that seen in 404B.


A
404A
20mV
sr stim
0.5sec
B
404B
20mV
sr stim
1sec

discharge rate was far below that seen in the same neurons during flight maintained by SR stimulation. Thus, a direct excitatory influence alone could not account for the maintained activity of the 404s.

Another possibility was that the central oscillator feeds back to excite the 404s and that the SRs, by increasing the oscillator frequency, increase the positive feedback to the 404s. Figure 62 shows the results of an experiment which argues against this possibility. Flight activity was accompanied by SR stimulation on every cycle. The SR stimulators were then turned off for approximately 50 cycles, following which the SR stimulation was recommenced. A constant windstream to the head was presented throughout. Turning off the SR stimulation resulted in no obvious change in 404 frequency although the rhythm generator frequency decreased 30%. Upon recommencing the SR stimulation the flight frequency increased at first rapidly and then more slowly. The response in the 404 (in this case a 404B) was an initial transient increase in frequency which quickly returned to its previous discharge rate even though the frequency of the rhythm continued to increase. Later in the same trial the SRs were again turned off. The frequency decreased rapidly with no change in the 404 frequency. However, once the the flight frequency reached approximately 6 Hz the 404 frequency then declined followed shortly after by cessation of the flight sequence. The results in Figure 62 were obtained with a B-type 404, which was only weakly excited by SR stimulation. However, the same result was also found for 404A (Fig.63).

The above shows that large changes in flight frequency can be induced by SR stimulation without producing a change in the 404 discharge rate. Hence, it does not seem likely that the central oscillator excites the 404s. These results also suggest that the maintained activity in the 404s is not due to an excitatory action from either the SRs or the central oscillator. Rather, it appears to be the wind stimulation to the head that is still responsible for the 404 activity. Figure 64 shows the response of the flight frequency and 404 discharge rate to turning off the windstream to the head. The SRs were stimulated on every cycle throughout the trial. When the wind was turned off, both the 404 discharge rate and the flight rhythm slowed down,

Figure 62. Changes in flight frequency evoked by SR stimulation are not accompanied by changes in activity in 404B. Upper graph: Average flight frequency versus time (average of every 5 flight cycles). Lower graph: 404 discharge rate versus time (average of every 20 spikes). Initially, the SRs were stimulated every flight cycle. At the times indicated the SR stimulation was turned off for 50 - 60 cycles. There was a large rapid drop in flight frequency but no change in the 404 discharge rate. Recommencing the SR stimulation caused at first a rapid, then a slow increase in flight frequency back to its previous levels but with no net change in 404 frequency. Later still the SR stimulation was terminated a second time. The flight frequency rapidly declined. Initially, there was no change in 404 activity. However, once the flight frequency fell to approximately 6 Hz then the 404 discharge rate also declined and the flight activity stopped.

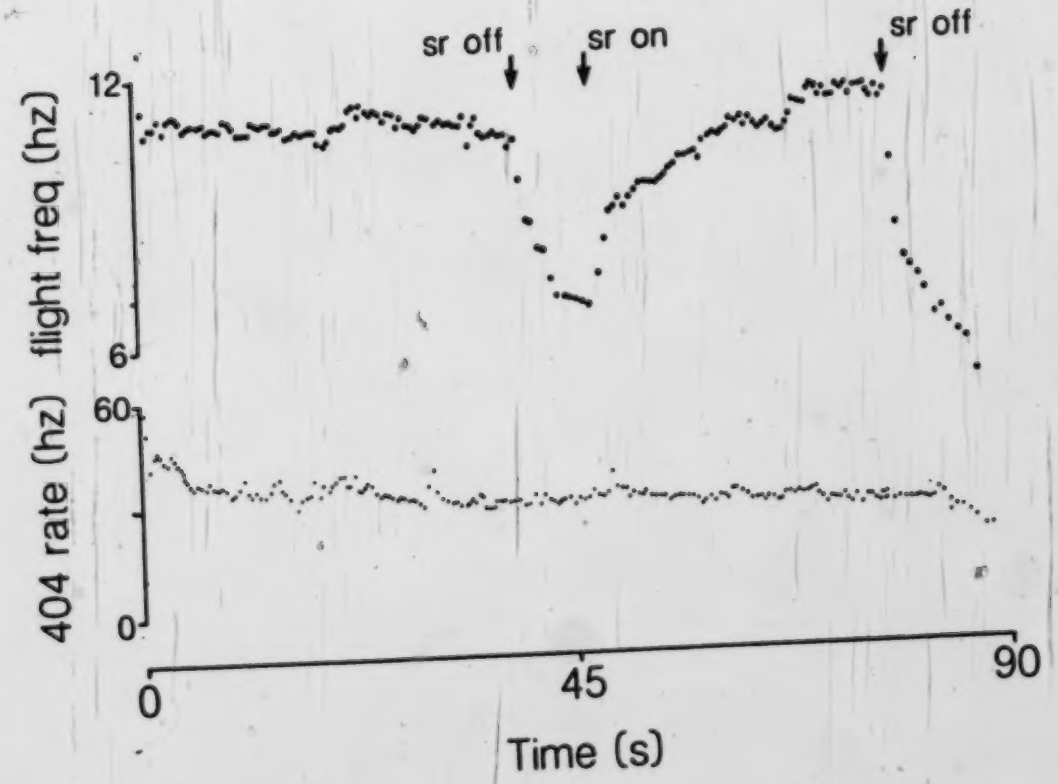

sr off
sr on
sr off
12
6
60
0
404 rate (hz) flight freq (hz)
0
45
90
Time (s)

Figure 63. Changes in flight frequency evoked by SR stimulation are not accompanied by changes in activity in 404A. Graphs as in previous figure. The experiment was the same as described in the previous figure except that the recording was made from an A-type 404.

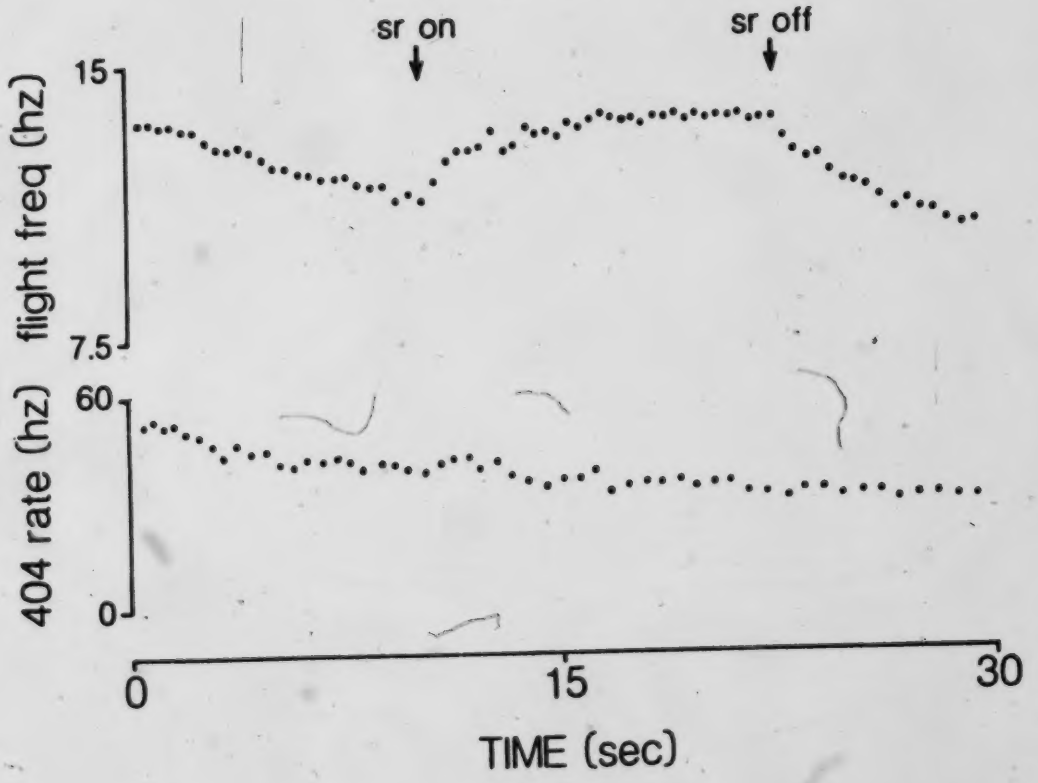
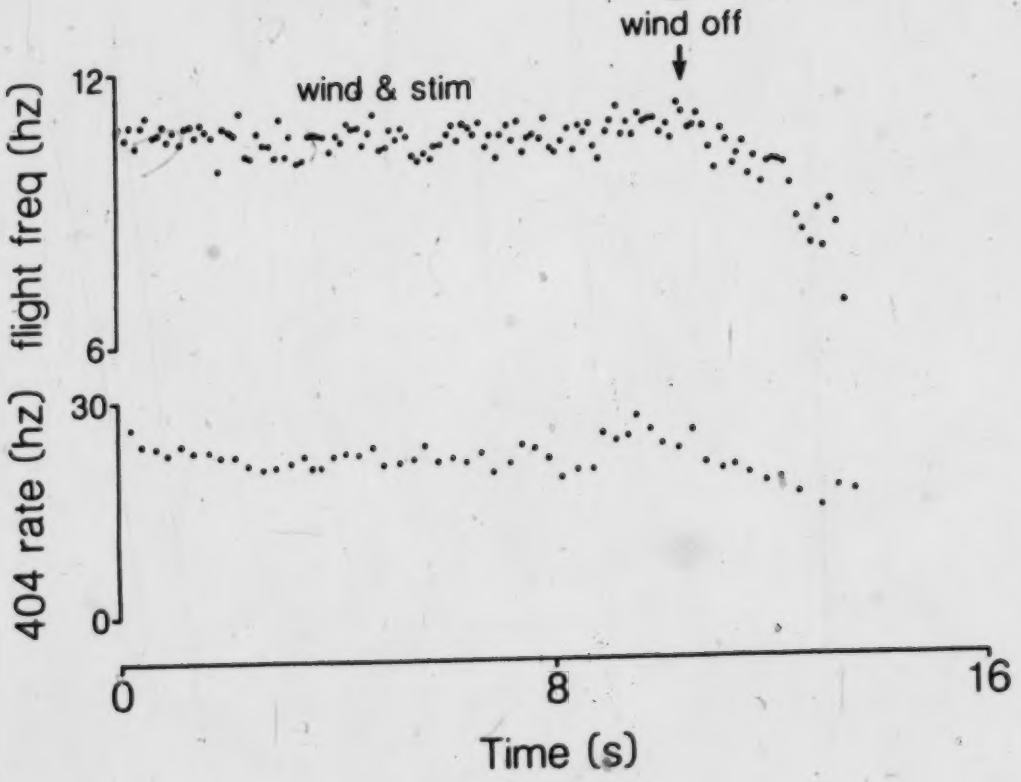

sr on
sr off
15
7.5
flight freq (hz)
60
0
404 rate (hz)
0
15
30
TIME (sec)

Figure 64. Effect of terminating a constant windstream to the head on the flight frequency and the 404 discharge rate. Upper graph: instantaneous flight frequency versus time Lower graph: average 404 discharge rate versus time (average of every 20 spikes). Data were taken from the end of a flight sequence. The origin of the time axis is arbitrarily set to zero. The forewing SRs were stimulated every cycle throughout the sequence shown. When the windstream on the locust's head was turned off, both the flight frequency and the 404 discharge rate declined and the flight activity stopped shortly thereafter.


wind off
wind & stim
12
6
flight freq (hz)
30
0
404 rate (hz)
0
8
16
Time (s)

followed shortly after by the end of the flight episode. What is somewhat surprising about this result is that in trials without SR stimulation and in those with SR stimulation, the wind stimulus to the head was identical. Yet without SR stimulation the wind kept the 404s active for only a short time (usually 5 - 10 seconds) while in the presence of SR stimulation the effectiveness of the wind stimulus was maintained for much longer times.

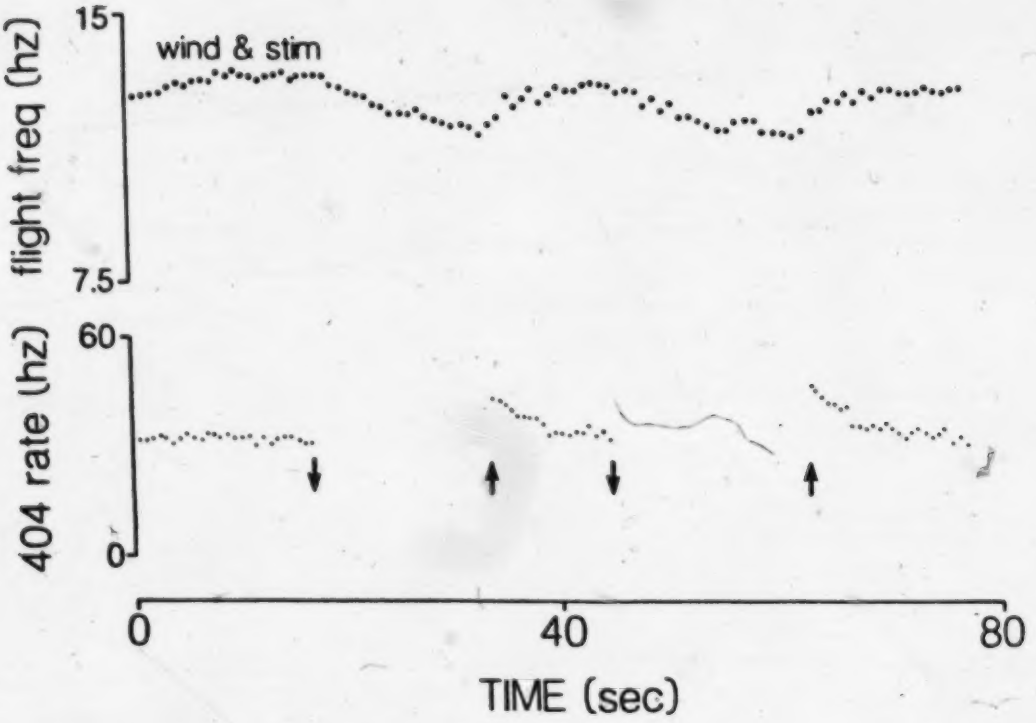
In summary, the above results show that the prolonged duration of flight sequences with SR stimulation is accompanied by prolongation of the activity in the 404s. This does not appear to be via a direct excitatory action of the SRs on these neurons but rather by an as yet unknown mechanism which maintains the effectiveness of descending wind interneurons in depolarizing the 404s.

The final question concerning the 404s addressed in this chapter is whether they play a role in the increase in flight frequency evoked by SR stimulation. Figures 62 and 63 show that this is not the case. Large increases and decreases in flight frequency can be induced by turning on and turning off the SR stimulation without affecting the 404 discharge rate. However, the 404s are still important determinants of the final flight frequency. Hyperpolarizing a single 404 during flight activity with accompanying SR stimulation still caused a reduction in flight frequency (Fig.65). Thus the final frequency of the flight rhythm will be determined not only by SR activity but also by the level of activity in the 404s.

D. DISCUSSION

This chapter described a group of mesothoracic interneurons whose properties suggested that they are responsible for initiating and maintaining the central flight rhythm. Ideally, three criteria should be met for the 404s to be ascribed this function (Kupfermann and Weiss 1978) : they should be active during the rhythm, mimicking their activity should initiate the rhythm (they are sufficient) and preventing their activity should prevent the expression of the rhythm (they are necessary). The 404s met the first two criteria. They discharged tonically throughout a flight sequence elicited by blowing wind on the head and depolarizing a single 404 in quiescent

Figure 65. Effect of 404 hyperpolarization on the flight rhythm with accompanying SR stimulation. Upper graph: average flight frequency versus time (average of every 10 cycles). Lower graph: average 404 discharge rate versus time (average of every 20 spikes). Flight activity was elicited by a constant windstream to the head and the SRs were stimulated immediately following the hindwing depressor spike on every cycle. At each downward arrow, the 404 was hyperpolarized to prevent action potentials. Each upward arrow marks the end of the hyperpolarizing pulse. Hyperpolarizing the 404 caused a slow decrease in the flight frequency. Conversely, upon releasing the hyperpolarization, the flight frequency increased to its former level.


flight freq (hz)
15
7.5
wind & stim
404 rate (hz)
60
0
0
40
80
TIME (sec)

animals initiated the flight rhythm. The third criterion is that they should be necessary for the expression of the flight rhythm. They were not necessary for flight activity elicited by touching the abdomen. None of the 404s were strongly activated by this stimulus and they do not appear to be involved in flight activity initiated in this fashion. However, the more important flight initiating pathway is that mediating information from the wind-sensitive hairs on the locust's head (Weis-Fogh 1949, 1956b). The 404s slowed or stopped ongoing flight activity when hyperpolarized (Fig.58) but could only sometimes prevent the initiation of flight by wind on the head. However, in these experiments only a single 404 was influenced and it is known that there are other 404s (at least 3 and possibly as many as 9 in the ganglion). The simplest explanation would be that the other 404s are not influenced by the hyperpolarization of one 404 and that they mediate excitation of the flight rhythm in this situation. Knowledge of possible synaptic or electrical connections between the different 404s is required before the adequacy of this argument can be assessed. Watson and Burrows (1983) have described the ultrastructure of neurons with very similar morphology in *Schistocerca gregaria*. They found that the neurites within the mesothoracic ganglion contained many synaptic output sites. The neurons receiving these synapses were not identified but one possibility is that some of these synapses are to other 404s.

Role of the 404s in the SR-induced increases in flight frequency

The 404s are important for determining the final frequency of the rhythm generator. Their firing rate is linearly related to the frequency of the flight rhythm generator (Fig.57). Hyperpolarizing a 404 slows the flight rhythm if there is or is not accompanying SR stimulation (Figs.58 and 65). However, the rapid increases in frequency caused by introducing SR stimulation are not due to an influence on the 404s. Large changes in flight frequency can be induced by turning the SR stimulation on and off, with no accompanying change in the 404 discharge rate. This is consistent with the conclusions reached in previous chapters that the SRs increase the flight frequency by a direct action on the central rhythm generator.

One question is whether the phase-independent increase in flight frequency reported by Wilson et al (Wilson and Gettrup 1963, Wilson and Wyman 1965) may be explained by an action of the SRs on the 404s. I have not been able to repeat these workers' results while recording from a 404 so the following is hypothetical. As was discussed in chapter three, the only conditions under which I have been able to repeat Wilson et al's findings of a slow, apparently phase-independent increase in frequency was by stimulating the SRs at a rate much higher than the oscillator frequency. The SRs do have an excitatory influence on the 404s in the quiescent state (Fig.61). One possibility is that with very high rates of SR stimulation the direct excitation to the 404s is of sufficient strength to increase the 404 discharge rate which would subsequently increase the frequency of the flight rhythm. As the 404s discharge tonically during flight, the timing of that stimulation in the flight cycle may be unimportant.

Recently, Möhl (1985c) has also argued in favour of a phase-independent pathway by which the SRs influence the flight rhythm. In his experiments, one SR nerve was stimulated in intact tethered flying animals. However, this SR nerve was left intact and the natural SR discharge was still occurring on each cycle. Therefore, when he stimulated the SR nerve on every cycle 2 SR bursts were transmitted to the nervous system - the natural SR discharge and the electrically evoked discharge. In his experiments, this would correspond to SR bursts repeated at the rate of 40 Hz (twice the flight frequency). Thus, it is possible that these very high rates of SR stimulation could increase the direct excitatory input to the 404s resulting in a decrease in period without the timing of that stimulation being important.

In principle, the 404s could act as a phase-independent pathway by which the SRs affect the frequency of the flight rhythm. However, the results from the second half of this chapter show that if the SR spikes are timed to occur at the flight frequency (the natural situation for a proprioceptor) then the 404s are not directly involved in the SR mediated changes in rhythm frequency. They are indirectly important however, in that it is

the 404s which are at least partly responsible for providing a tonic drive to the oscillator, which then determines the intrinsic frequency of the flight rhythm. The level to which SR stimulation increases the flight frequency will then depend on the level of activity in the 404s.

Is there an Hierarchical Organization in the Locust Flight System?

The data presented in the first half of this chapter suggested that the locust flight system was hierarchically organized. Wind interneurons descending from the brain excite the 404s which then provide a tonic drive to the central rhythm generator. Davis and Kovac (1981) suggested that, in general, rhythmic motor systems are not hierarchically organized and that command neurons receive excitatory feedback from the rhythm generator. Several observations suggest it is unlikely that the 404s receive positive feedback from the central rhythm generator. These are:

(i) there is no marked rhythmic modulation of the 404s

(ii) it is possible to activate the central rhythm generator (by touching the abdomen) without strongly exciting the 404s (Fig.59).

(iii) introduction or cessation of SR stimulation causes large changes in oscillator frequency with no change in the 404 discharge rate (Figs.62 and 63).

Thus these results support the idea that the locust flight system is hierarchically organized. However, the results presented in the second half of the chapter suggest that the organization of the locust flight system may be more complex than a simple unidirectional hierarchy. These experiments showed that with SR stimulation during flight the 404s maintained a steady discharge rate. Neither a direct excitatory influence of the SRs on the 404s nor positive feedback from the oscillator (see above) can account for the maintained discharge of the 404s. However, turning off the windstream caused both the 404 discharge rate and flight frequency to drop and flight sequence to stop shortly afterward. The question that still remains to be answered is why does a constant windstream to the head fail to keep the 404s firing when there is no SR stimulation, yet the same wind stimulus can

maintain 404 activity for long periods of time if there is accompanying SR stimulation. In Figures 60 and 62, it can be seen that the 404 discharge rate appears to decline sharply once the flight frequency declines to around 6 Hz. Indeed it is extremely rare in these preparations that animals maintain such a low frequency. It appears that if the flight oscillator frequency falls below this level then descending input from the wind hairs is no longer capable of keeping the 404s active. The ability of the SRs to prolong the activity in the 404s may be due to the fact that they increase the flight oscillator frequency, thereby keeping it above this critical level.

VI. DISCUSSION

A. Phase-dependent Influence on the CPG

The first section of this thesis examined the hypothesis put forward by Wilson and Gettrup (1963) that phasic proprioceptors can have purely phase-independent influences on a central pattern generator. Stimulating one pair of SRs in deafferented locusts, these workers found that the flight frequency increased slowly with no phase preference shown between the stimulus and motor output (Wilson and Gettrup 1963, Wilson and Wyman 1965). I showed in the first chapter that the SRs had strong phase-dependent effects on the timing of the flight rhythm in semi-deafferented preparations. This was demonstrated in several ways. Firstly, if SR stimulation was triggered on every cycle immediately following the hindwing depressor spike, there was a rapid increase in frequency beginning on the first cycle of stimulation and the duration of the flight sequence was prolonged. These effects were not observed with stimulation occurring approximately midway between cycles. Secondly, SR stimuli given at different times in the flight cycle caused a phase-dependent advance in the flight rhythm if the stimuli fell close to the depressor phase. Thirdly, the flight rhythm could be entrained by SR stimulation. These influences of the SRs on the flight rhythm indicated that they could act as peripheral timing cues for the central rhythm generator. The question arises of why did Wilson and Gettrup (1963) and Wilson and Wyman (1965) fail to see phase-dependent effects of SR stimulation in their experiments? Several pieces of data related to this point came from experiments described in other chapters. The major differences between the experiments of chapter two and those of the Wilson, Gettrup and Wyman were the extent of deafferentation, the site of stimulation of the SR afferent and the particular pair of SRs stimulated (ipsilateral or segmental). In chapter three it was shown that both segmental pairs of SRs (forewing or hindwing) could entrain the deafferented flight rhythm. The ability to do this was not influenced by which peripheral branch of the SR afferent was stimulated. On the other hand, in only one exceptional case was entrainment seen if a lateral pair of SRs was stimulated. This was

the SR pair stimulated by Wilson et al. Hence this appears to largely explain their negative findings. However, by triggering the SR stimulation from the motor output a phase-dependence could still be demonstrated. Wilson *et al* did not perform this test. One reason that a lateral pair of SRs cannot entrain the rhythm may be related to the cellular studies presented in chapter four. There it was found that most SR connections are only to neurons ipsilateral to the SR afferent. Therefore, many of the central interneurons receiving SR input would not be influenced by the stimulation. The slow increase in frequency reported by Wilson et al required high rates of SR stimulation in my preparations. In chapter five I showed that some of the 404s received an excitatory input from the SRs. Thus, the slow increase in frequency may be in part due to increased 404 activity. However, if the SRs were triggered to occur on every flight cycle then no change was detected in the 404 discharge rate. It appears, therefore, that the 404s do not play a role in increasing flight frequency in the preparation that I used, in which the maximum frequencies reached were approximately 12/s. Perhaps in the intact animal, where there are four SRs active, bursting at a rate of 20/s the SRs do influence the flight frequency via the 404 pathway.

In summary, these results showed that the wing SRs do have phase-dependent influences on the locust flight rhythm generator and no conclusive evidence for phase-independent effects was found. There is no evidence from other systems of purely phase-independent effects of a phasic proprioceptor. Therefore, there is now no experimental support for this concept.

There are now a number of behaviors where proprioceptors have been demonstrated to influence the timing of a rhythm generator. The SRs in locust flight could act as a timing cue for the CPG by signalling when the wing reaches the top of the wing upstroke. Analagous situations where receptors which signal the end of one phase of a movement feed back to the rhythm generator are found in other systems. For example, in the cat, blocking leg extension during the stance phase prevents the initiation of a flexion movement (Grillner and Rossignol 1978). However, if the leg is permitted to move to its normal posterior position then a flexion

will abruptly ensue. A similar reflex operates at the transition from flexion to extension (Andersson et al 1981). The responsible afferents appear to arise from the hip (Andersson and Grillner 1981, 1983). In the lamprey, signals from stretch receptors within the spinal cord act on the CPG to limit the amplitude of the movements (Grillner et al 1981). However, in cat walking and swimming in the lamprey and the dogfish, input to the rhythm generators not only comes at the end of the movement. Imposed movements with an amplitude smaller than the normal range can also entrain these rhythm generators (Grillner et al 1981, Grillner and Wallén 1982, Andersson and Grillner 1983). This suggests that the rhythm generators are also sensitive to the direction of movement.

The influences of peripheral timing cues on rhythm generators appears to vary between different behaviors. The phase-dependence for the SRs' effects on the flight rhythm was primarily one of advances around depressor phase with no effect occurring midway in the cycle. Although I cannot exclude a delaying influence, the slow rate at which entrainment occurred when the stimulus rate was less than the intrinsic frequency indicates this must be weak. In walking and respiration in the cat, swimming in the dogfish and the lamprey, proprioceptive stimulation can both advance and delay the central rhythm generators (Andersson and Grillner 1981, 1983, Feldman and Gautier 1976, Grillner and Wallén 1982, Grillner et al 1981). Phase response curves from proprioceptors which show a predominance of advancing phases have been reported in the feeding systems of the lobster (Nagy and Moulins 1981) and *Limax* (Beltz and Gelperin 1980). However, in the case of the lobster, a small delaying influence was also seen. The SRs are well known to be important in establishing the intact flight frequency (Wilson and Gettrup 1963, Kutsch 1974). The SRs fire at the top of the wing upstroke (Möhl 1985b), close to the depressor phase. This is in the advancing region of the flight cycle. Hence the flight frequency is increased. An analagous situation occurs in cat respiration where the Hering-Breuer reflex terminates inspiration and is responsible for the shorter period seen in respiration in the intact animal (Clark and von Euler 1972).

Proprioceptors are not usually considered as part of the rhythm generator. However, there is no reason to justify the exclusion of the SRs from the locust flight rhythm generator. They meet all the commonly used criteria for inclusion in the rhythm generator. They discharge rhythmically in phase with the motor output, the motor output is altered when they are removed and they can reset the flight rhythm. They are not necessary for rhythm generation *per se*. However, this is also true for many central neurons, which nonetheless are still included as elements of the central rhythm generator. For example, it was shown in the pyloric rhythm of the lobster stomatogastric ganglion that no single neuron or group of neurons were necessary for generation of a rhythmic motor output Miller and Selverston 1982). In addition, while the SRs may not be necessary for rhythm generation they are important in the maintenance of flight activity.

What functions do the SRs have in the generation of flight? They are clearly important in establishing the intact flight frequency. However, this is not due to a slow excitatory influence but, rather, to its advancing action on the central rhythm generator. Wendler (1974) suggested that the wing proprioceptors also stabilize the frequency. Kutsch (1974) noted an increase in the cycle-to-cycle variability of period after lesioning the wing proprioceptors. The entrainment experiments in chapters two and three showed that the SRs can modulate the period of the flight rhythm. Therefore they may also be involved in this function. Another clear role of the SRs is in maintaining flight activity. The duration of deafferented flight sequences is usually very short whereas in the presence of SR stimulation the duration increases markedly. Some insights into the mechanisms by which this occurs was gained from the study of the 404 neurons. This is discussed in more detail later in this chapter. A fourth role for the SRs is in controlling the amplitude of the wingstroke. However, as shown in chapter four, a more complex mechanism is involved than the simple negative feedback scheme of Burrows (1975). The SRs make short-latency connections to depressor motoneurons and appear able to act in a negative feedback loop to limit the wing elevation (Möhl 1985b, 1985c). However, the other part of Burrows'(1975) negative feedback loop (turning off the elevator burst) would not seem

important based on behavioral considerations. This is because the SRs do not fire until well after the elevator motoneurons have ceased firing (Möhl 1985b). Furthermore, I found no evidence to suggest a short-latency inhibition from the SRs to the elevator motoneurons. In fast movements, time constraints limit the ability of sensory feeback to elicit compensatory responses to a perturbation during the performance of that movement (Rack 1981). One way around this problem is the use of anticipatory or predictive control (Arbib 1981, Rack 1981). For example, in the production of human speech sounds, a brief downward perturbation of the lower lip produces a compensatory lowering of the upper lip so that labial closure may be achieved (Abbs and Gracco 1984, Gracco and Abbs 1985). The amplitude of this response depends on the time in the movement that the perturbation is given (Gracco and Abbs 1985). In locust flight, the control of wingbeat amplitude seems to rely in large part on a predictive process. The amount of depolarization seen in both elevators and depressors at the top of one wingstroke is strongly dependent on the firing of the SRs from the top of the previous wingstroke. I have only studied this phenomenon with regards to the presence or absence of SR input. My results indicate that if a particular wing elevation was weak so that there was little or no SR activity then the following wing elevation will be stronger due to removal of the inhibitory influence of the SRs on the elevator motoneuron. However, these influences require much more study. One would like to know their dependence on phase angle, frequency and duration of the SR burst. In addition, both ipsilateral and contralateral forewing SRs produce these effects. Therefore, there seems to be a dependence on the movements of the other wings as well. A fifth function of the SRs in locust flight, then, may be interwing co-ordination. There are several indications of this. As described, the SRs produce long latency influences on both ipsilateral and contralateral elevator and depressor motoneurons. The SRs connect to premotor interneurons which project to contralateral motoneurons. Also, there is convergence of SR information onto many interneurons. Indeed, wherever tested, all interneurons receiving SR input did so from at least two SRs.

B. Modification of Interneuronal Activity by Proprioceptors

To help understand the cellular mechanisms by which proprioceptors modify the flight CPG, I have identified many interneurons which receive short-latency PSPs from the SR afferents. These were all excitatory PSPs. Only one neuron responded to single SR spikes with an inhibitory PSP but this was at a considerably longer latency than the EPSPs seen in other neurons. EPSPs from the wing SRs were found in motoneurons, premotor interneurons, a rhythm generator interneuron and flight initiating interneurons. Thus, the SRs' influence is widespread throughout the pattern generator.

The most interesting result from this study was the degree to which the SRs could modify the centrally generated rhythmic activity of particular interneurons. An increased frequency of discharge and/or an increase in burst duration was observed in some interneurons. The SRs also evoked EPSPs in elevator interneurons. In one of these I showed that SR stimulation changed its rhythmic pattern markedly so that it fired mainly as a depressor interneuron. SR stimulation abolished rhythmic depolarizations in one interneuron and also provided a phasic input to a neuron which was not driven by the rhythm generator. In the cat respiratory system, the influence of proprioceptors on the rhythmic activity of central neurons has been examined in considerable detail. During normal respiration in the cat, inspiratory activity causes lung inflation which excites pulmonary stretch receptors (PSR) in the bronchioles (von Euler 1981). The PSRs are responsible for the Hering-Breuer reflex which causes an abrupt termination of inspiratory activity. Interestingly, some of the same features of proprioceptive modulation observed in the respiratory system were found to occur in locust flight.

The majority of SR connections were to interneurons active in phase with depressors. This was expected as the SRs normally fire close to this phase of the flight cycle (Wilson and Gettrup 1963, Möhl 1979, 1985b). Similarly, in cat respiration the PSRs fire maximally toward the end of lung inflation and excite late inspiratory neurons (Berger 1977, Marino et al 1981, Cohen and Feldman 1984). Also neurons active early in expiration have been found to be

excited by lung inflation (Feldman and Cohen 1978). In the locust, neuron 124 is excited early in elevation and receives a strong SR input which would be expected to advance its firing in the flight cycle.

In the locust, I identified one interneuron (519) which did not receive central flight oscillator input yet it received a phasic input from the wing stretch receptors. In the nucleus of the solitary tract in the cat brainstem the 'pump' neurons similarly do not show respiratory modulation in the absence of artificial ventilation (Berger 1977, Lipski et al 1979, Cohen and Feldman 1984). These units are equally excited by lung inflations during inspiration or expiration (Cohen and Feldman 1984). Berger (1977) showed that the pump neurons were excited by stimulation of the superior laryngeal nerve, establishing that they were not primary afferent fibres from the lungs.

In another locust interneuron (308), SR stimulation on every cycle abolished its rhythmic depolarizations. A similar phenomenon has also been reported in pontine respiratory neurons by Feldman et al (1976). Here, neurons were found which had only low tonic background activity when the animal was artificially ventilated but rhythmic modulation of their discharge would appear if lung inflation was witheld. However, for 308 in the locust, I have shown that other wing afferents excite this cell and therefore there is probably a phase-shift of its activity dependent on proprioceptive input, rather than it being functionally inactive during intact flight.

The degree of modulation of rhythmic interneuronal activity by proprioceptive feedback in locust flight raises the question of what is the central pattern generator? I have looked at the input from only one group of proprioceptors. Phasic sensory inputs also come from the head hairs (Bacon and Möhl 1979, Horsmann et al 1983), the tegulae (Kien and Altman 1979, Neumann 1985) and the wing campaniform sensillae (Gettrup 1966, Horsmann and Wendler 1985). One would expect that the activity of many interneurons in intact animals will be considerably altered , including large phase-shifts and additional central neurons being active.

In its common use, the term central pattern generator carries with it the implication of being a hardwired functional unit. For example, Grillner (1975; see also Andersson et al 1981, Grillner and Wallén 1985) feel that the cat CPG within the spinal cord would be sufficient to generate walking in a straight line on a flat surface. Proprioceptors would only gain prominence when turning, accelerating, decelerating, walking uphill or downhill. This is similar to the early view taken by Wilson (1961) in the locust flight system. He felt that all that was required was a tonic excitation to increase the speed of the flight CPG. Certainly, the capacity to oscillate resides within the CNS. However, it seems distinctly possible that when the proprioceptive feedback is incorporated one will not be able to recognize a distinct central pattern generator. An analogy may be drawn with the experiments of Miller and Selverston (1982) in the lobster stomatogastric system. These workers inactivated particular neurons involved in generating the pyloric rhythm. No individual neuron was necessary for rhythm generation but the phase relations between neurons were altered. Recent work in this system has also shown that neuromodulators can have dramatic effects on the durations of bursts and phase relations between neurons (Nagy and Dickinson 1983, Marder 1984). In the same vein, the same circuit used for generating the swim rhythm in *Tritonia* can also generate a reflexive withdrawal depending on the level of activity in a particular interneuron (Getting and Dekin 1985). What these results suggest is that the same neural circuits responsible for pattern generation may function in different ways. For locust flight it may be that quite fundamental changes are induced in the activity of the flight pattern generator by proprioceptive feedback. Obviously, evaluating this possibility will depend on understanding how the pattern generator functions in both the deafferented and intact animals.

C. Maintenance of Rhythmic Activity by Proprioceptors

In the course of this study, more information has been obtained on the central organization of locust flight. Specifically, this was related to the mechanisms of initiation and maintenance of the central rhythm (chapter five).

There are only a few examples of single neurons which, when depolarized, can initiate a rhythmic behavior and which have also been demonstrated to be active in that behavior. These are the 204 and 205 neurons in leech swimming (Weeks and Kristan 1978, Weeks 1982), the C2 and DSI neurons in *Tritonia* swimming (Getting 1977, Lennard et al 1980), the PCN neurons in *Pleurobranchaea* feeding (Gillette et al 1978) and the SO neurons in *Lymnaea* feeding (Rose and Benjamin 1981). All of these neurons display some degree of rhythmic activity and in the case of the DSI, C2, PCN, and SO and possibly also the leech 205 neurons, may be considered part of the rhythm generator network. As a consequence, Davis and Kovac (1981) have suggested that the concept of a separate neuronal system providing an unpatterned input to a rhythm generator may not be true. Rather, they propose that there are reciprocal excitatory connections between command and rhythm generator systems.

In chapter five, I described a set of interneurons which appear to be responsible for the initiation and maintenance of locust flight (the 404s). They fired tonically throughout a flight sequence initiated by blowing wind on the head with a discharge rate that was linearly related to the flight frequency. Intracellular injection of depolarizing currents into a single 404 was sufficient to initiate flight activity. Hyperpolarizing a single 404 could inhibit the initiation of flight sometimes but not in all cases. Similarly, hyperpolarizing a 404 during ongoing flight slowed the flight rhythm and occasionally stopped it. Thus it is not firmly established that the 404s are necessary for flight initiated by wind on the head. However, this is likely related to the fact that only one 404 in a population of 404s was being influenced. However, the flight rhythm could be elicited by touching the abdomen without strongly exciting the 404s. Therefore, other neurons must exist which are also involved in flight initiation.

In comparison with other systems, the 404s have a close resemblance to the 204 neurons which can initiate swimming in the leech (Weeks and Kristan 1978). These neurons discharge throughout swim episodes and can evoke the swimming rhythm when depolarized (Weeks and Kristan 1978). As the frequency of the swimming rhythm declines so does the mean discharge rate of the 204s. Hyperpolarizing two 204s could not prevent the initiation of swimming.

However, passing depolarizing or hyperpolarizing currents into a 204 would increase or decrease the swimming frequency respectively (Weeks and Kristan 1978, Weeks 1982). The one major difference between the leech 204s and 404s in locust were that the former were more obviously modulated in phase with the swimming rhythm. Thus the 204s must receive some feedback from the swim oscillator. However, Weeks (1981) has suggested that any phasic information from the 204s to the oscillator must be weak if it exists. She was able to show this by eliciting swimming in two ganglia which were only joined by a connective which contained the 204 axon but not the axons of swim oscillator interneurons. Each ganglion produced a rhythmic swim motor output but these were randomly phased with respect to one another, suggesting little, if any, phasic information in the 204 discharge. The 404s in locust were observed in some cases to receive a weak inhibition in phase with depressors. However, this was very small and any functional significance seems unlikely. The data in both the leech and the locust support the concept of a separate neuronal system conveying an essentially unpatterned input to a central rhythm generator.

A unidirectional hierarchical chain has often been considered to underlie the central organization of rhythmic movements. The initial observations on the 404s suggested that this may be true for locust flight (Pearson et al 1985). However, this must be revised after considering the effects of proprioceptive stimulation on 404 activity. In chapters two and three, it was shown that the SRs prolong the duration of flight sequences in response to wind on the head. This was shown to be accompanied by prolonged activity in the 404s. The windstream applied to the head was the same in both conditions (i.e. with and without SR stimulation) and, in addition, the wind stimulus was still necessary to maintain flight activity. Therefore, there must be some feedback from the oscillator or the SRs to the 404s. However, a phasic excitatory influence from either the SRs or the oscillator was ruled out. Clearly, the maintenance of flight involves mechanisms more complex than a simple hierarchy or the reciprocal excitatory scheme of Davis and Kovac (1981). Despite any clear conclusions regarding mechanism, my results do show that the wing SRs are important in the maintenance

of activity in the flight rhythm generator.

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